L'inflammation hépatique dans les formes sévères de NAFLD : implications cliniques, médiateurs et stratégies diagnostiques

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Sujet de la thèse

L'inflammation hépatique dans les formes sévères de NAFLD: implications cliniques, médiateurs et stratégies diagnostiques

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Dédicace

Pour exprimer son âme, on n'a que son visage ….
Jean Cocteau, Renaud et Armide

Je dédie cette thèse à mes parents
en guise de témoignage d'amour,
respect
et reconnaissance…

Remerciements

Soyons reconnaissants aux personnes qui nous donnent

du bonheur ; elles sont les charmants jardiniers

par qui nos âmes sont fleuries.

Marcel Proust

C'est à la fin de cette longue démarche couvrant les 4 années de thèse qu'on écrit ce chapitre, « les remerciements ». C'est la partie la plus agréable mais aussi la plus difficile à exprimer car les mots manquent aux émotions… C'est un moment charnier de rétrospection mais aussi d'introspection et d'analyse critique du chemin parcouru avec un certain recul face à soi même.

On se rend compte que « *Nous n'arrivons pas toujours à changer les choses suivant notre désir, mais que peu à peu notre désir change*", que "*Nos plus grandes craintes, comme nos plus grandes espérances, ne sont pas au-dessus de nos forces, et nous pouvons finir par dominer les unes et réaliser les autres*" (Marcel Proust).

C'est aussi le moment ou on se rend compte aux mieux que ce travail, loin d'être solitaire n'aurait jamais été possible sans le soutien de près ou de loin des nombreuses personnes dont l'intérêt, la générosité et les bons conseils étaient essentielles pour avancer.

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la persévérance, de la détermination, de l'excellence dans le travail et de l'exigence face à soi même.

« Le plus grand danger pour la plupart d'entre nous n'est pas que notre but soit trop élevé et que nous le manquions mais qu'il soit trop bas et que nous l'atteignons », Michel-Ange.

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Enfin, « *L'amitié est une création de l'homme, la plus haute de toutes qui comporte la clairvoyance, qui admet les défauts … »* (Jean Cocteau), au final pour « *aimer que ce qu'on ne possède pas tout entier* » (Marcel Proust). Je remercie tous mes collègues et mes ami(e)s, nouveaux et anciennes qui ont été a mes cotés, m'ont encouragé, soutenu et

accompagné pendant les moments de haut et de bas. "*Les relations les plus solides s'établissent toujours sur l'envie qu'on a de les faire durer, et la connaissance exacte de ce qui les menace*", Francis Scott Fitzgerald.

Je remercie celui qui est maintenant un ami depuis plus de 20 ans, qui m'a donnée la liberté faisant qu'il y a 10 ans exactement ma carrière a basculé de la Roumanie vers la France. «*Amitié qui finit n 'avait point commencé*», Publius Syrus

Les mots les plus simples étant les plus forts je les adresse à mes parents pour exprimer mon affection et ma reconnaissance. Malgré mon absence et mon éloignement depuis si longtemps, votre confiance et votre amour m'ont toujours guidé les pas. Je vous doit pleinement tout ce que je suis aujourd'hui. Je sais que vous aurez aimé être ici, ce jour, mais rassurez vous, malgré votre absence vous êtes là, remplissant pleinement mon cœur. Merci !

Vivre est la chose la plus rare.

La plupart des gens se contents

d'exister.

Oscar Wilde.

Liste des Abréviations

AGL : Acides gras libres

AST : Aspartat amino transferase

ALT : Alanin amino transferase

AMPK : AMP-activated protein kinase

APRI : AST to **P**latelet **R**atio **I**ndex

BCLC :

CHC : Carcinome Hépatocellulaire

CK18 : Caspase Generated Keratin 18 Fragment

CV : Cardiovasculaire

EIMc : Epaisseur intima-media carotidienne

FIB-4 : Fibrosis-4 Index

FGF-19 : Fibroblast **G**rowth **F**actor 19

FLI : Fatty **L**iver **I**ndex

FT : Fibrotest

FS : FibroScan (elastometrie transitionelle)

FXR : Farnesoide X receptor

GGT : gamma glutamyl transpeptidase

GWAS : Genetic **W**ide **A**ssociation **S**tudies

HDL: High density lipoprotein

HOMA-IR : Homeostasis Model Assessment

IGF: Insulin Growth Factor

IL-6 : Interleukine 6

IMC : Index de Masse Corporelle

IR : Insulinorésistance

IR-A : Insulin Receptor A

IR-B : Insulin Receptor B

IRM : Imagerie par résonance magnétique

IRS : Insulin Receptor Substrat

JNK : c-Jun N terminal kinase

kPa : kiloPascales

LDL : Low density lipoprotein

LPS : Lypopolysaccharides

LXR : Liver X receptors (récepteurs des oxystérols)

MAPK : Mitogen-Activated Protein Kinase

MCP-1 : Monocyte Chemotactic Protein 1

mTOR : Mammalian Target of Rapamycin

NAFL : Stéatose isolée (non-alcoholic fatty liver)

NAFLD : Stéatose hépatique non-alcoolique (**N**on-**A**lcoholic **F**atty **L**iver **D**isease)

NAHANES III : National Health and Nutrition Examination Survey III

NAS : NAFLD **A**ctivity **S**core

NASH : Steatohépatite non-alcoolique (**N**on-**A**lcoholic **S**teato**H**epatitis)

NF-kB : Nuclear factor kappa B

NLRP3 : Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 dependent inflammasome

NLRP6 : Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-6 dependent inflammasome

PAMPs : Pathogen-associated molecular patterns

PBH : Ponction Biopsie Hépatique

PPARs : Peroxisome Proliferator-Activated Receptors

PTEN : Phosphatase and TENsin homolog

SAF : Steatosis **A**ctivity **F**ibrosis

SOCS : Supressor of Cytokine Signaling

SREBP : Sterol **R**egulatory **E**lement **B**inding **P**rotein 1c

STAT3 : Signal Transducer and Activator of Transcription 3

TGR 5 : Transmembrane G protein-coupled receptor

TLR 4 : Toll-like Receptors 4

TM6SF2 : Transmembrane 6 superfamily member 2

TNF alpha : Tumor Necrosis Factor alpha

VIH : Virus de l'immunodéficience humaine

VLDL : Very low density lipoprotein

VHB : Virus de l'hépatite B

VHC : Virus de l'hépatite C

Résumé

La stéatose hépatique non-alcoolique (non- alcoholic fatty liver disease - NAFLD) regroupant la stéatose isolée (NAFL) et la steatohépatite (NASH) est une des causes le plus fréquentes des maladies chroniques du foie et devient un enjeu majeur responsable d'une augmentation considérable des dépenses de santé publique. Compte tenu de la prévalence croissante de l'obésité et du diabète ainsi que des avancées récentes permettant le traitement et le contrôle de l'hépatite C, l'importance et la place de la NAFLD en hépatologie va encore augmenter.

La dichotomie entre la stéatose et la steatohépatite est décisive pour la stratification du risque évolutif. La stéatose isolée présente un faible risque de progression tandis que les patients atteints de steatohépatite sont à risque de mortalité et morbidité hépatique (développement de la cirrhose, du carcinome hépatocellulaire) et extra hépatiques (cardiovasculaire, rénale).

Malgré beaucoup de progrès réalisés ces dernières années, les mécanismes physiopathologiques de la maladie responsables de la grande diversité phénotypique (NASH avec poids normal ou associée à l'obésité) et des différences pronostiques à long-terme (stéatose isolée, progression vers la NASH, cirrhose et ses complications) sont encore insuffisamment connues. L'inflammation chronique systémique semble avoir un rôle majeur dans la progression de lésions hépatiques et le développement des complications extra hépatiques.

L'objectif général de ce travail était de mieux définir le rôle de l'inflammation hépatique dans l'histoire naturelle de la NAFLD à travers des études cliniques permettant d'analyser : (1) les facteurs associés avec l'évolution des lésions histologiques, (2) l'impact de la stéatose et des facteurs de risque métabolique sur le

développement du carcinome hépatocellulaire chez les patients déjà au stade de cirrhose; (3) l'impact de la stéatose sur la survenue à long-terme de lésions précoces d'athérosclérose.

Dans une première étude nous avons analysé dans une cohorte des patients atteints de la NAFLD avec biopsies de suivi à long-terme les facteurs associés à la progression des lésions hépatiques : transition de la NAFL vers la NASH et progression de la fibrose. Nous avons démontré que les lésions d'inflammation lobulaire ou de fibrose, même minimes, sont associées avec un risque de progression de la maladie à moyen terme. Souvent cette progression s'accompagnait d'une aggravation des facteurs de risque métabolique. Par conséquent, ces malades doivent bénéficier d'un suivi rapproché et d'une prise en charge spécifique par mesures générales (perte du poids, régime hygiéno-diététique, activité physique) ou traitement pharmacologique (y compris inclusions dans les essais cliniques). Ces résultats, si confirmés, permettront de réaliser une meilleure stratification du risque évolutif de la maladie et suggèrent que les pratiques cliniques actuelles de prise en charge des malades atteints de la NAFLD doivent être reconsidérés.

Dans la deuxième étude nous avons analysé la prévalence et l'impact de la stéatose et des facteurs de risque métabolique sur le risque de développer un carcinome hépatocellulaire chez des malades avec cirrhose alcoolique ayant subi une transplantation hépatique. Nous avons démontré que les facteurs de risque métabolique sont fréquents chez les patients avec une maladie alcoolique du foie et augmentent significativement le risque de carcinome hépatocellulaire même au stade de cirrhose. Ces résultats permettent d'identifier un groupe des patients ayant un risque élevé de carcinome hépatocellulaire. Ces malades pourraient justifier des procédures renforcées de surveillance et de dépistage. On peut spéculer que le contrôle des facteurs de risque

métabolique pourrait contribuer à réduire le risque de carcinome hépatocellulaire. Des études incluant tout le spectre de la maladie alcoolique du foie doivent être effectuées afin de mieux analyser l'interaction entre la consommation d'alcool et les facteurs de risque métabolique sur la progression de la fibrose, le développement de la cirrhose et le risque de carcinome hépatocellulaire.

Dans la troisième étude nous avons exploré la prévalence de la NAFLD dans une cohorte des patients suivis dans un centre de prévention cardiovasculaire primaire, dans le but d'analyser l'impact de la stéatose sur la sévérité et l'incidence des lésions précoces d'athérosclérose carotidienne. Dans un premier temps nous avons démontré dans une large cohorte transversale de plus de 5000 patients ayant deux ou plusieurs facteurs de risque cardiovasculaire que la stéatose était un facteur indépendant associée avec la sévérité des lésions d'athérosclérose au delà de l'association fréquente avec les facteurs de risque cardiovasculaire classiques. Dans un deuxième temps, dans une cohorte de 1800 patients avec un suivi moyen de 8 ans, nous avons démontré que la stéatose était associée avec la survenue de lésions d'athérosclérose carotidienne durant la période de suivi. Ces résultats, si confirmés, suggèrent que la stéatose est non seulement un marqueur mais un facteur qui intervient dans la pathogenèse de l'athérosclérose carotidienne, permettant ainsi une meilleure stratification du risque cardiovasculaire. Cela implique que les patients atteints de la NAFLD doivent bénéficier d'une évaluation initiale du risque cardiovasculaire. On peut aussi spéculer que le traitement de la NAFLD pourrait réduire le risque cardiovasculaire. Des études prospectives chez des patients avec NAFLD prouvée histologiquement sont nécessaires pour confirmer nos résultats.

En conclusion, ce travail nous a permis d'explorer les facteurs et les mécanismes potentiels responsables de la progression de la maladie hépatique, le développement du carcinome hépatocellulaire et l'augmentation du risque cardiovasculaire. L'inflammation hépatique, dans un contexte de stéatose, pourrait favoriser l'expression de médiateurs pro-athérogènes et l'activation des voies de carcinogenèse ce qui aurait pour effet de favoriser l'apparition des complications extrahépatiques, c'est à dire métaboliques, cardio-vasculaires et néoplasiques chez les patients avec NAFLD. Des études cliniques prospectives prenant en compte les facteurs environnementaux, cliniques, génétiques et le microbiome intestinal sont nécessaires afin de permettre une meilleure stratification du risque de patients atteints de la NAFLD.

Summary

Non-alcoholic fatty liver disease (NAFLD) is becoming one of the most frequent causes of chronic liver diseases worldwide, responsible for a significant increase in public health costs. Because of the increasing prevalence of obesity and type-two diabetes and the recent advances allowing the cure of chronic hepatitis C, these trends will probably continue to increase and NAFLD will become the leading cause of chronic liver disease in the years to come.

The spectrum of NAFLD encompasses two different entities with different clinical course and different prognosis: simple steatosis (NAFL) and steatohepatitis (NASH). The classical view is that patients with NAFL have a benign clinical course, while those with NASH have a significant increase in overall and specific morbidity and mortality.

Despite the considerable increase in knowledge over the past decade there is still uncertainty about the precise mechanisms that drive the clinical diversity (different clinical phenotypes – lean or obese NAFLD) and the clinical course of the disease (simple steatosis, NASH, development of cirrhosis, hepatocellular carcinoma). The low-grade chronic inflammation seems to be the driving force accounting for the disease progression and development of hepatic and extra hepatic complications.

The overall aim of this work was to better understand the role of chronic systemic inflammation into the natural history of NAFLD and to analyze: (1) the factors associated with the progression of histological lesions and the transition from simple steatosis to NASH; (2) the prevalence and impact of steatosis and metabolic risk factors on the development of hepatocellular carcinoma in patients with cirrhosis; (3) the impact of steatosis on the long-term incidence of early carotid atherosclerosis.

We first undertook a study of NAFLD patients with repeat liver biopsies in order

to better understand the histological course of the disease, specifically in relation to the initial findings of NAFL or steatohepatitis. We also aimed at correlating histological changes to changes in the metabolic co-morbidities often associated with NAFLD. In this report, which is one of the largest monocentric series of carefully defined patients with NAFL, we show that disease progression towards unambiguous steatohepatitis (severe ballooning) and bridging fibrosis is possible, even after a rather short follow- up of less than 5 years. We demonstrated that mild lobular or portal inflammation or fibrosis in any location substantially increases the risk of progression to steatohepatitis or advanced fibrosis. Disease progression often occurred concomitant with worsening of the metabolic conditions during follow-up. Consequently and pending confirmation by future reports, current practices of no or very relaxed hepatic monitoring in patients with steatosis alone might not be appropriate, and a more active, hepatological follow-up could be necessary.

In the second study, we have analyzed the prevalence and the impact of liver steatosis and metabolic risk factors on the risk of developing hepatocellular carcinoma in patients with alcoholic cirrhosis undergoing liver transplantation. The main finding of this study was that patients with advanced ALD have a high prevalence of NAFLD, and that this comorbid association confers a significantly increased risk of hepatocellular carcinoma. These findings are important for risk stratification of HCC in patients with ALD. It can be speculated that controlling for metabolic risk factors may result in a decreased risk of hepatocellular carcinoma. Further studies including patients with the whole spectrum of alcoholic liver disease should be performed to confirm our findings and analyze the magnitude of the interaction between alcohol consumption and metabolic risk factors on fibrosis progression, development of cirrhosis, and

hepatocellular carcinoma.

In the third study we examined the impact of liver steatosis on the presence and progression of carotid intima-media thickness (C-IMT) and carotid plaques (CP) in a large transversal and longitudinal follow-up cohort of patients with two or more cardiovascular risk factors seen in a Primary Prevention Center. We first demonstrated in a transversal study (> than 5000 patients) that steatosis predicted carotid atherosclerosis independently of the association with classical cardiovascular risk factors. Second, in a subset of patients with longitudinal follow-up (>1800 patients and mean follow-up of 8 years) we demonstrated that baseline NAFLD was an independent predictor for incident carotid plaques. These results suggest that NAFLD is not only a marker but also an "active player" in the pathogenesis of atherosclerosis, allowing for a better stratification of cardiovascular risk. Patients with NAFLD should have an initial evaluation of cardiovascular risk. Treating NAFLD may reduce the cardiovascular risk. Future prospective studies in patients with histologically defined NAFLD are required to confirm our results.

In conclusion, this work analyzed the potential factors involved in the progression of histological lesions in NASH, development of hepatocellular carcinoma and increased cardiovascular risk. Low-grade chronic inflammation responsible for the production of pro-atherogenic cytokines and the activation of pro-oncogenic signaling pathways might represent the link between liver fibrosis progression, hepatocellular carcinoma development and increased cardiovascular risk. Future prospective studies are required to analyze the interaction between clinical, environmental and genetic factors and to allow a better risk stratification of NAFLD patients.

Liste de publications en relation avec la thèse

Article # 1. Article original, publie en Septembre 2013, **Journal of Hepatology - IF 11.33**

A systematic review of follow-up biopsies reveals disease progression in patients with non- alcoholic fatty liver.

Raluca Pais, Fréderic Charlotte, Larysa Fedchuk, Pierre Bedossa, Pascal Lebray, Thierry Poynard, Vlad Ratziu, for the LIDO Study Group. Journal of Hepatology 2013 Sep; 59(3):550-6. doi: 10.1016/j.jhep.2013.04.027.

Article # 2. Article original, publié en May 2015, **Clinical Gastroenterology and Hepatology – IF 7.89**

Nonalcoholic Fatty Liver Disease Increases the Risk of Hepatocellular Carcinoma in Patients With Alcohol-Associated Cirrhosis Awaiting Liver Transplants

Raluca Pais, Pascal Lebray, Geraldine Rousseau, Frédéric Charlotte, Ghizlaine Esselma, Eric Savier, Dominique Thabut, Marika Rudler, Daniel Eyraud, Corinne Vezinet, Jean-Michel Siksik, Jean-Christophe Vaillant, Laurent Hannoun, Thierry Poynard, and Vlad Ratziu. Clin Gastroenterol Hepatol. 2015 May;13(5):992-9.e2. doi: 10.1016/j.cgh.2014.10.011. Epub 2014 Oct 20.

Article # 3. Article original, soumis, **Journal of Hepatology – IF 11.33**

Fatty Liver Is An Independent Predictor Of Early Carotid Atherosclerosis: Results From a Large Transversal Study and Long-Term Follow-Up

Raluca Pais, Philippe Giral, Jean-François Khan, David Rosenbaum, Chantal Housset, Thierry Poynard and Vlad Ratziu for the LIDO Study Group.

Articles en annexe :

Article # 4. Revue, publié en Septembre 2013 – **Journal of Hepatology – IF 11.33**

From NAFLD in clinical practice to answers from guidelines

Nascimbeni F, **Pais R**, Bellentani S, Day CP, Ratziu V, Loria P, Lonardo A. J Hepatol. 2013 Oct;59(4):859-71. doi: 10.1016/j.jhep.2013.05.044. Epub 2013 Jun 7.

Article # 5. Article original publié en Novembre 2014, **Alimentary Pharmacology and Therapeutics – IF 5.72**

Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease.

Fedchuk L, Nascimbeni F, **Pais R**, Charlotte F, Housset C, Ratziu V; LIDO Study Group. Aliment Pharmacol Ther. 2014 Nov;40(10):1209-22. doi: 10.1111/apt.12963. Epub 2014 Sep 29.

Article # 6. Article original publié en Janvier 2015, **European Journal of Internal Medicine – IF 2.3**

Prevalence of steatosis and insulin resistance in patients with chronic hepatitis B compared with chronic hepatitis C and non-alcoholic fatty liver disease.

Pais R, Rusu E, Zilisteanu D, Circiumaru A, Micu L, Voiculescu M, Poynard T, Ratziu V. Eur J Intern Med. 2015 Jan;26(1):30-6. doi: 10.1016/j.ejim.2014.12.001. Epub 2014 Dec 29.

Article #7. Revue, publié en Février 2014, **Clinics in Liver Disease – IF 2.7**

The impact of obesity and metabolic syndrome on chronic hepatitis B and drug-induced liver disease.

Pais R, Rusu E, Ratziu V. Clin Liver Dis. 2014 Feb;18(1):165-78. doi: 10.1016/j.cld.2013.09.015.

Objectifs de la thèse

Malgré une forte prévalence dans la population générale et beaucoup des progrès récents les mécanismes et les facteurs de risque responsables de la progression de la NAFL vers la NASH, la progression de la fibrose et le développement des complications hépatiques (cirrhose, carcinome hépatocellulaire) et extra-hépatiques (cardiovasculaires, rénales, etc.) sont mal connus.

Notre hypothèse de travail était que l'état inflammatoire chronique associé à la NAFLD serait responsable non seulement pour la progression de la maladie hépatique mais aussi du développement des complications extra-hépatiques.

L'accumulation des triglycérides dans le foie est une réponse physiologique adaptative à l'augmentation d'apport calorique et est actuellement considérée plus un marqueur qu'une cause de la résistance à l'insuline qui ne serait pas hépatotoxique en soi [1]. En revanche, la lipotoxicité induite par les acides gras libres (AGLs) a un rôle majeur dans l'inflammation hépatique et la progression de la NAFL vers la NASH[2]. Les AGLs sont responsables non seulement du stress oxydant avec production de radicaux libres, mais aussi de l'activation des TLRs (toll-like receptors), de la production de cytokines et de chimiokines pro-inflammatoires (TNFα, IL-1β, IL-6), et de la lipoapotose. Cette réaction inflammatoire spécifiquement hépatique qui s'accompagne également d'une libération des médiateurs pro-athérogènes pourrait expliquer l'association fréquente entre les NAFLD et les maladies cardiovasculaires. L'inflammation hépatique précède parfois les lésions de stéatose et semble être le principal facteur responsable de la progression de la fibrose et l'évolution vers la cirrhose et ses complications[3]. Le stress oxydatif et l'activation des voies de signalisation NF-kB dépendantes entretiennent l'état inflammatoire chronique propre aux NAFLD et interviennent dans la progression de la fibrose et dans le développement tumoral[4].

L'objectif général de ce travail était de mieux définir le rôle de l'inflammation hépatique dans l'histoire naturelle des NAFLD à travers des études cliniques permettant d'analyser : (1) les facteurs associés avec l'évolution des lésions histologiques, la transition entre la stéatose isolée et la NASH et la progression de la fibrose chez des malades atteints de NAFLD avec biopsies de suivi a long-terme; (2) l'impact de la stéatose et des facteurs de risque métabolique sur le développement du carcinome hépatocellulaire chez les patients déjà au stade de cirrhose; (3) l'impact de la stéatose sur la survenue à long-terme des lésions précoces d'athérosclérose afin de déterminer si la NAFLD est un marqueur ou un facteur de risque indépendant d'atteinte cardiovasculaire.

Chapitre I. Stéatose hépatique non–alcoolique (NAFLD)

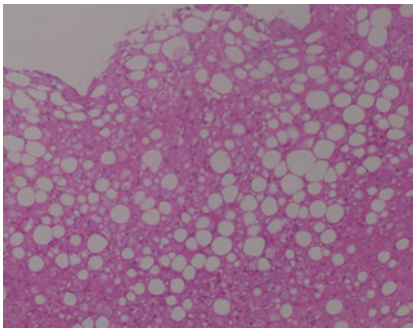
I-1. Définition

Historiquement, la stéatose non alcoolique du foie (le terme anglais-non-alcoholic fatty liver disease, NAFLD) a été décrite pour la première fois en 1980 par Ludwig et ses collaborateurs comme une entité reproduisant les lésions histologiques de l'hépatite alcoolique et qui peut évoluer vers la cirrhose. Les auteurs ont observé que cette maladie mal connue touche surtout les femmes et s'associe souvent avec l'obésité et le diabète de type 2[5].

La définition actuelle des NAFLD s'appuie sur des critères histologiques, cliniques et pronostiques. La NAFLD regroupe deux entités distinctes : la stéatose isolée (stéatose touchant plus de 5% des hépatocytes sans lésions de nécroinflammation et sans fibrose) et la stéatohépatite, (stéatose touchant plus de > 5% des hépatocytes avec des lésions d'inflammation lobulaire ou de ballonisation hépatocytaire avec ou sans fibrose)[6] (**Figure 1)**.

Figure 1. Définition histologique de la NAFLD. Stéatose isolée sans lésions de nécroinflammation (A, Hematoxyline-eosine) et sans fibrose (B, Rouge Sirius). Steatohépatite (NASH) : stéatose >5% avec lésions d'inflammation lobulaire et de ballonisation hepatocytaire (C, Hematoxyline-eosine) et fibrose portale (D, rouge Sirius).

A.



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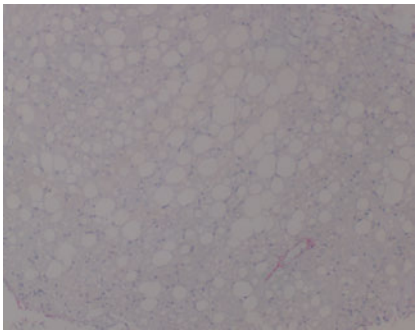
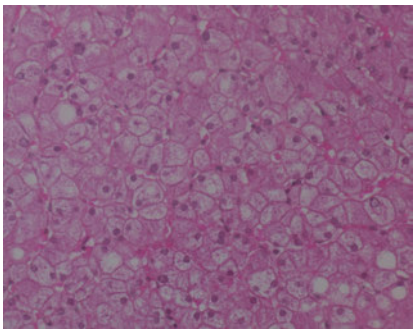


C.



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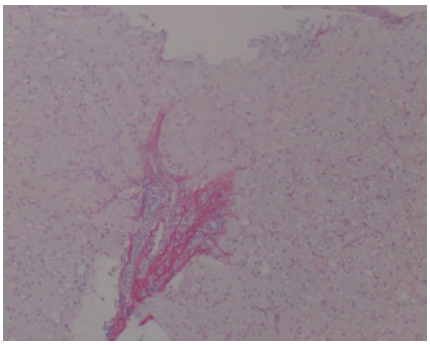


Les sociétés savantes Européenne et Américaine ont complété cette définition histologique par l'affirmation « en absence d'autre cause de maladie chronique du foie – telle que le virus de l'hépatite B ou C, l'hépatite auto-immune, la consommation excessive de l'alcool, la maladie de Wilson, l'hémochromatose, le déficit en alpha 1 antitrypsine, ou les médicaments connus hépatotoxiques »[7, 8].

La NAFLD devient ainsi un diagnostic d'exclusion, après avoir éliminé les autres causes de maladie chronique du foie. Les deux sociétés savantes définissent la

« consommation excessive d'alcool » par un seuil de 20 g/jour pour les femmes et 30 g/jour pour les hommes et respectivement de 210 g/semaine pour les hommes et 140 g/semaine pour les femmes[7, 8]. Des études épidémiologiques suggèrent que la stéatose induite par l'alcool devient significative au delà de ces seuils[9]. Toutefois, plusieurs études ont montré que la NAFLD pouvait coexister avec les hépatites virales ou la maladie alcoolique du foie parfois en aggravant leur evolution[10-13].

L'avancée majeure de ces dix dernières années est la reconnaissance de l'appartenance de cette maladie au cadre nosologique de l'insulinorésistance (IR) et de ses complications phénotypiques (surpoids, diabète de type 2, dyslipidémie etc). On considère actuellement que la NAFLD représente la manifestation hépatique du syndrome métabolique ce qui implique la recherche de cette affection en pratique clinique chez les patients à risque.

Ces arguments sont en faveur d'un changement de nom abandonnant le qualificatif « *non-alcoolique en absence d'autres causes de maladie chronique du foie* » pour le terme « *métabolique* » reconnaissant ainsi le caractère indépendant de la maladie, la coexistence avec d'autres maladies du foie et le rôle de l'insulinorésistance et des facteurs de risque métabolique comme facteurs causals.

Rarement, la NAFLD est secondaire à d'autres causes dans l'absence de l'insulinorésistance. (**Tableau 1**).

Tableau 1. Causes secondaires de NAFLD.

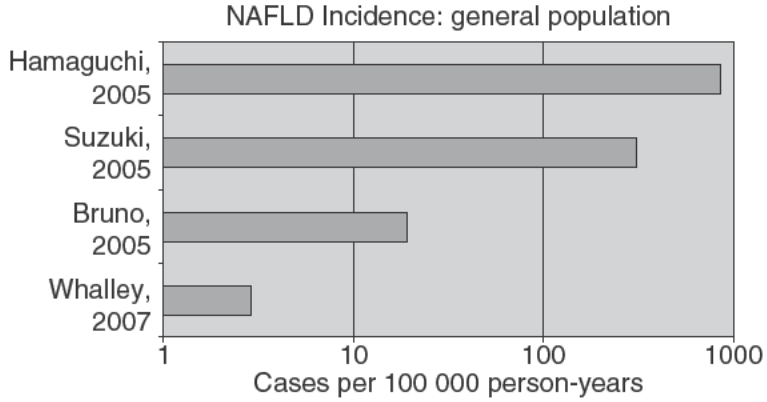
■ **Maladies génétiques ou métaboliques**
Maladie de Wilson
Hypo-betalipoproteinemie
Syndromes lipodystrophiques
Maladie de Weber- Christian
Maladie de Wolman
Maladie de dépôt des esters de cholestérol
■ **Toxines industrielles**
■ **Nutritionnelles/Chirurgicales**
Bypass Jejuno-ileal
Nutrition parentérale totale
Jeune prolongé
Malnutrition protéique
■ **Médicaments**
Corticostéroïdes
Tamoxifene
Inhibiteurs calciques
Amiodarone
Estrogènes
Tetracyclines
Anti-retroviraux anti –VIH

I-2. Epidémiologie

I-2. 1. Incidence

L'incidence de la NAFLD reste mal connue. Les études prospectives où la NAFLD était diagnostiquée à l'échographie, ont retrouvé une incidence allant de 18.5 à 86 pour 1000 sujets par an[14, 15], ce qui reflète l'hétérogénéité des populations étudiées mais aussi la variabilité de la durée de suivi (**Figure 2**).

Figure 2. Incidence de NAFLD dans la population générale (Vernon et al.[16]).



I-2.2. Prévalence

La prévalence de la NAFLD varie beaucoup en fonction de la région géographique et de la méthode diagnostique employée.

Il est actuellement estimé que 20–25% de la population adulte au niveau mondial a une stéatose[17]. Aux Etats-Unis, il est estimé qu'un tiers de la population adulte a une stéatose[18]. La prévalence de la NAFLD en Europe et au Moyen Orient varie entre 20% et 30%[19-21]. Des études récentes au Japon et en Chine retrouvent une prévalence de la NAFLD similaire à celle retrouvée Europe (20%-30% au Japon et 15%-30% en Chine)[22, 23]. En raison de l'adoption d'un mode de vie sédentaire et des habitudes diététiques de pays de l'Occidentaux, la prévalence de la NAFLD a significativement augmenté dans les pays en cours de développement. La prévalence de la NAFLD dans les régions urbaines

de l'Inde est semblable à celle de l'Europe et varie entre 16% et 32%[24, 25]. Toutefois, dans les régions rurales pauvres de l'Inde elle est beaucoup plus faible (9%)[26]. Le niveau socio-économique, le mode de vie sédentaire et l'adoption des habitudes diététiques des pays occidentaux, « fast-food diet », expliquent les principales différences de prévalence entre les pays développés et en cours de développement : (1) prévalence sensiblement plus élevée dans les pays de l'Ouest (20–30% en Occident vs. 10–20% dans les pays de l'Est) ; (2) prévalence plus élevée dans les zones urbaines que dans les zones rurales dans les pays en cours de développement alors que ces différences sont effacés dans les pays de l'Ouest; (3) prévalence plus faible de l'obésité dans les pays en cours de développement mais plus élevée de la NAFLD chez les sujets non obèses dans ces pays[27].

Il existe des variations importantes dans la prévalence rapportée de la NAFLD en fonction de la méthode diagnostique utilisée.

La plupart des études dans la population générale ont utilisé comme méthode diagnostique soit l'échographie soit l'élévation des transaminases. Néanmoins, les deux méthodes sous-estiment la prévalence réelle de la NAFLD. Il est connu que l'échographie a une sensibilité limitée pour la détection de la stéatose inférieure à 30%[28]. D'autre part, une proportion significative de patients avec une NAFLD ont des transaminases normales[29].

Une étude récente réalisée aux Etats-Unis parmi des sujets consultant à l'hôpital pour affections non-hépatologiques a retrouvé une prévalence de la stéatose de 46%, de la stéatohépatite (confirmée histologiquement) de 12% et de la fibrose avancée de 2.7%[30].

Une étude anglaise réalisée chez des sujets sans facteurs de risque de maladie chronique du foie, vus en médecine générale pour élévation des transaminases, a

retrouvé une prévalence de la stéatose à l'échographie de 26% et de 1.6% de la fibrose avancée évaluée par des marqueurs sériques[31].

La prévalence de la stéatose évaluée par des techniques nouvelles de spectroscopie par résonance magnétique serait de 30% chez les Américains et les Chinois[32, 33].

La NAFLD diagnostiquée par biopsie hépatique chez les patients sélectionnés comme donneurs vivants pour une transplantation hépatique (considérés comme sujets sains) est de 3–16% en Europe et aux Etats Unis[34-37].

Les études provenant des centres tertiaires de référence retrouvent une prévalence plus élevée des NAFLD et de la NASH parmi les sujets consultant pour transaminases élevées ou stéatose à l'échographie. Dans une étude multicentrique portant sur 272 sujets explorés par biopsie hépatique, la prévalence de la NAFLD était de 59%, dont 27% avec stéatose simple, 32% avec stéatohépatite et 26% avec une fibrose avancée[38].

I – 3. Facteurs de risque

I-3.1. Age.

La prévalence de la NAFLD augmente avec l'âge et est supérieure à 40% chez les sujets âgées de 70 ans ou plus qui sont le plus à risque de développer une fibrose ou une cirrhose (prévalence de 40% et 14% respectivement)[39]. L'addition des facteurs de risque métaboliques, notamment cardiovasculaires, la dysfonction mitochondriale et la redistribution du tissu adipeux, sont des facteurs qui augmentent l'insulinorésistance et qui expliquent la prévalence élevée de la NAFLD chez les personnes âgées. Toutefois, elle semble diminuer chez les sujets octogénaires (21% après 85 ans vs 36-39% avant 85 ans), ayant survécu à la mortalité liée aux comorbidités associées à la stéatopathie[40].

I-3.2. Sexe.

Une large étude effectuée à Hong-Kong a retrouvé une augmentation significative de la prévalence de la NAFLD chez les femmes de plus de 50 ans[33]. Ces résultats pourraient suggérer un rôle des hormones sexuelles dans la pathogenèse de la maladie. Cette hypothèse est confirmée par la prévalence élevée de la NAFLD dans le syndrome des ovaires polykystiques[41].

I-3.3. L'obésité et les facteurs de risque métabolique.

La résistance à l'insuline a un rôle majeur dans la pathogenèse de la NAFLD et explique les liens étroits entre la NAFLD, le syndrome métabolique et ses composantes, particulièrement l'obésité et le diabète. La NAFLD est actuellement considérée comme la manifestation hépatique du syndrome métabolique[42]. Dans une analyse récente du registre américain NHANES III, la prévalence du syndrome métabolique parmi les patients avec NAFLD était de 79%[43].

Les études issues des cohortes de chirurgie bariatrique chez les obèses morbides ont rapporté une prévalence élevée de la NAFLD allant jusqu'à 90% dont 37% pour la NASH [44]. Toutefois, les sujets non obèses provenant des pays en cours de développement ont une prévalence de la stéatose de 10–20%, dont 30% ont une NASH et 2.5% une cirrhose[45]. Ces patients sans obésité apparente (IMC <25 kg/m^2), ont néanmoins une adiposité viscérale excessive et une insulinorésistance.

Chez les patients diabétiques de type 2 la prévalence de la NAFLD est de 70%.

Le **Tableau 2** résume la prévalence de la NAFLD en fonction de la présence des différents facteurs de risque métabolique.

Tableau 2. Prévalence de la stéatose à l'échographie chez les sujets à risque pour la NAFLD.

Facteurs de risque	Prévalence
Obésité	90%
Diabète de type 2	70%
Dyslipidémie	50%
Syndrome métabolique	59%
Syndrome d'ovaires polykystiques	42%

I-3.4. L'ethnie et les facteurs génétiques.

La prévalence de la NAFLD varie considérablement en fonction de l'ethnie et s'accompagne souvent des phénotypes cliniques particuliers. Des études récentes ont décrit une prévalence élevée de la résistance à l'insuline (59%) ainsi qu'une augmentation du contenu hépatique en triglycérides de 2 à 3 fois chez les Indiens asiatiques de la côte Est des Etats-Unis comparativement aux sujets caucasiens[46]. Des

études ont trouvé une prévalence élevée de la NAFLD chez les sujets d'origine hispanique aux Etats Unis. En revanche, les sujets africains, malgré un taux d'obésité et de diabète élevé semblent être protégés de la NAFLD[18].

Ces différences de prévalence inter-ethniques ne peuvent pas être attribuées uniquement aux différences de niveau socio-économique, culturel ou de mode de vie (niveau d'activité physique, habitudes alimentaires) et soulignent le rôle potentiel des facteurs génétiques.

Parmi les facteurs génétiques, le mieux étudié est le polymorphisme du gène de l'adiponutrine (PNPLA3 – *adiponutrine/patatin-like phospholipase 3*). Les sujets présentant un polymorphisme GG rs738409 de l'adiponutrine ont 3 fois plus de chances d'avoir une stéatose, une stéatohépatite ou une fibrose avancée indépendamment de la présence des éléments du syndrome métabolique[47]. Ainsi, ce polymorphisme, le plus constamment retrouvé par les études de GWAS (*genetic wide association studies*), semble jouer un rôle important non seulement dans le risque de développer une stéatose mais également dans le risque de survenue des formes plus sévères de la maladie.

I-4. Pathogenèse

La pathogenèse de la NAFLD est un processus complexe qui implique l'interaction entre des mécanismes multiples, expliquant le caractère « systémique » de la maladie qui dépasse largement le foie. Il est essentiel de comprendre les mécanismes responsables du développement et de la progression de la maladie car cela permet non seulement d'identifier les patients à risque mais aussi d'identifier des cibles thérapeutiques potentielles.

I-4.1. Accumulation de triglycérides dans le foie et lipotoxicité.

Historiquement, le modèle de survenue de la NAFLD/NASH est le « *2-hit hypothesis* » décrit par Day et al., en 1998 : l'accumulation des lipides dans le foie était le « 1st hit » et les phénomènes de stress oxydatif et beta-oxydation le « 2nd hit », responsable de l'apparition des lésions de nécroinflammation[48]. Cette hypothèse est actuellement de plus en plus remise en question. Des études ultérieures ont démontré que l'accumulation de triglycérides dans le foie est plus un marqueur de l'insulinorésistance et représente plutôt une réponse adaptative à l'augmentation du flux des acides gras libres (AGL) vers le foie[2]. En revanche les métabolites toxiques des AGL sont responsables de la lipotoxicité et de l'apparition de lésions de nécroinflammation. Les mécanismes intervenant dans la lipotoxicité qui sont responsables de l'apparition des lésions d'inflammation et nécrose hépatocytaire sont résumées dans la **Figure 3**.

La lipotoxicité est souvent concomitante à l'accumulation de triglycérides dans le foie, mais les deux phénomènes sont indépendants les uns des autres[1]. Des études

expérimentales confirment cette hypothèse et montrent que l'inactivation de la diacyglycerole acyltransferase 2 (DGAT2), l'enzyme finale qui catalyse la formation des triglycérides, induit une diminution du contenu hépatique en triglycérides mais ne prévient pas les lésions de lipotoxicité induites par les métabolites toxiques d'AGL[49].

La résistance à l'insuline est la principale conséquence de dysfonction du tissu adipeux et favorise l'hypertrophie des adipocytes et leur infiltration macrophagique. Dans ce contexte les adipocytes présentent un altération du profil sécrétoire des adipocytokines pro et anti-inflammatoires avec un effet net favorisant l'inflammation locale et systémique[50]. L'hypoadiponectinemie est corrélée avec la sévérité des lésions histologiques dans la NASH indépendamment de l'insulinorésistance[51]. Les voies de signalisation NF-kB (nuclear factor kappa B) et JNK (c-Jun N terminal kinase) ont un rôle important dans l'inflammation.

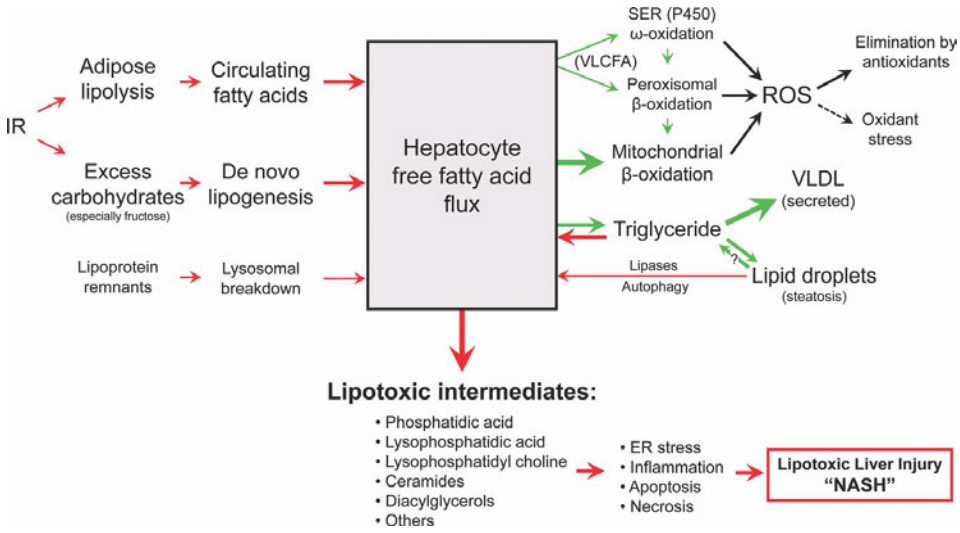
D'autres part, l'état inflammatoire chronique associée à la NAFLD favorise et entretient la résistance à l'insuline[3, 52]. La résistance à l'insuline périphérique est une des causes les plus importantes de lipolyse du tissu adipeux responsable de l'augmentation du flux des AGL vers le foie, les dépôts de lipides et l'inactivation de certains facteurs de transcription tels récepteurs PPAR gamma (Peroxisome Proliferator-Activated Receptors)[53]. Au niveau hépatique la résistance à l'insuline inhibe la gluconéogenèse.

Une autre source d'AGL est la lipogenèse hépatique *de novo* favorisée par la surexpression de certains facteurs de transcription tels que sterol regulatory element binding protein 1c (SREBP-1c)[54] ou l'apport excessive de fructose[55].

Les AGL en excès sont dégradés par beta-oxydation mitochondriale ou ré-estérifiés en TG et ensuite éliminés en tant que VLDL. Le déséquilibre entre la beta-oxydation mitochondriale et l'export de triglycérides par la voie des VLDL favorise

l'accumulation des métabolites toxiques (céramides, diacyglycerols)[56]. Les métabolites toxiques des AGL favorisent le stress oxydatif et l'apoptose conduisant à l'apparition des lésions de lipotoxicité (inflammation et nécrose hépatocytaire)[57].

Figure 3. Les principaux mécanismes intervenant dans la lipotoxicité (Neuschwander-Tetri et al.)[1]



I 4. 2. Rôle des récepteurs nucléaires (PPARs et FXRs)

La superfamille des récepteurs nucléaires regroupe des facteurs de transcription de nature protéique qui sont activés par un ligand et se fixent sur des séquences d'ADN spécifiques, à proximité des gènes qu'ils régulent, pour en modifier l'activité transcriptionnelle. Les récepteurs PPARs, FXRs et LXRs font partie de la famille 1 des récepteurs nucleaires[58].

Les récepteurs PPARs ont un rôle majeur dans le contrôle du métabolisme lipidique, la différenciation cellulaire et l'inflammation tissulaire et interviennent comme facteurs clés dans le syndrome métabolique et les affections cardiovasculaires[59].

Les récepteurs PPARα sont exprimés dans les tissus présentant une forte activité de catabolisme des AG tels que le foie, le muscle squelettique et le cœur. Les principales fonctions des récepteurs PPARα sont : la régulation du métabolisme des lipides et lipoprotéines, l'homéostasie des lipides dans les macrophages et le contrôle de la réponse inflammatoire[59].

Les récepteurs PPARδ sont exprimés dans des nombreux tissus. Les principales fonctions des récepteurs PPARδ sont: l'augmentation de la beta-oxydation des AGL ; l'inhibition de la lipogenèse hépatique (via l'inhibition de SREBP 1c) ; la réduction de la gluconéogenèse hépatique (via l'activation de l'AMPK) ; l'amélioration de l'inflammation hépatique (via l'inhibition du STAT3). Les récepteurs PPARδ ont aussi un effet hépatoprotecteur via l'inhibition de la phosphorylation de la JNK et la modulation de l'activité inflammatoire des macrophages[60, 61]. Des modèles expérimentaux ont aussi démontré un effet antifibrosant des récepteurs PPARδ[62].

Compte-tenu des effets sus décrits des récepteurs PPARα/PPARδ il existe un rationnel puissant pour tester les doubles agonistes PPARα/PPARδ dans la NAFLD. Des études préliminaires ont démontré que le GFT505, un double agoniste PPARα/PPARδ améliore l'insulinorésistance hépatique et périphérique, la dyslipidémie, l'inflammation hépatique et les transaminases[63, 64].

Les récepteurs PPARγ sont exprimés essentiellement au niveau du tissu adipeux et à faible niveau dans le muscle, le foie et le rein. Les principaux effets des récepteurs PPARγ sont résumés dans le **Tableau 3**[65].

Les agonistes pharmacologiques de PPARγ (les glitazones) augmentent la sensibilité à l'insuline et exercent un effet anti-inflammatoire, hépatoprotecteur et

diminuent l'activation des cellules étoilées. La résolution de la NASH a été observée chez 47% des patients traités avec pioglitazone pendant 2 ans[66].

Tableau 3. Synthèse des principaux effets résultant d'une stimulation des récepteurs PPARγ

- **Effet sur le tissu adipeux :**
  - stimulation de l'adipogenèse
  - redistribution de la masse grasse
  - diminution de la libération des AGL
  - diminution de la production de TNF alpha
  - augmentation de la production d'adiponectine
- **Effet sur le muscle squelettique :**
  - augmentation de la sensibilité à l'insuline
  - augmentation de l'utilisation de glucose
- **Effet sur le foie :**
  - diminution de la stéatose
  - augmentation de la sensibilité à l'insuline
  - diminution de la production de glucose
  - modification du profil lipidique

Les récepteurs FXRs. Des études récentes montrent que les acides biliaires ont un rôle important dans le contrôle du métabolisme glucidique et lipidique, souvent en conjonction avec le microbiote intestinal et les incretines[67]. Leur action est modulée par les récepteurs nucléaires, dont les plus important sont les récepteurs FXR (farnesoid X receptor) et les récepteurs TGR5 (transmembrane G protein-coupled receptor). Les récepteurs FXR sont activés par les acides biliaires via une boucle de contre-réaction (negative feedback loop) qui sert à contrôler la production des acides biliaires et de maintenir un niveau stable dans l'intestin par rapport aux besoins[68].

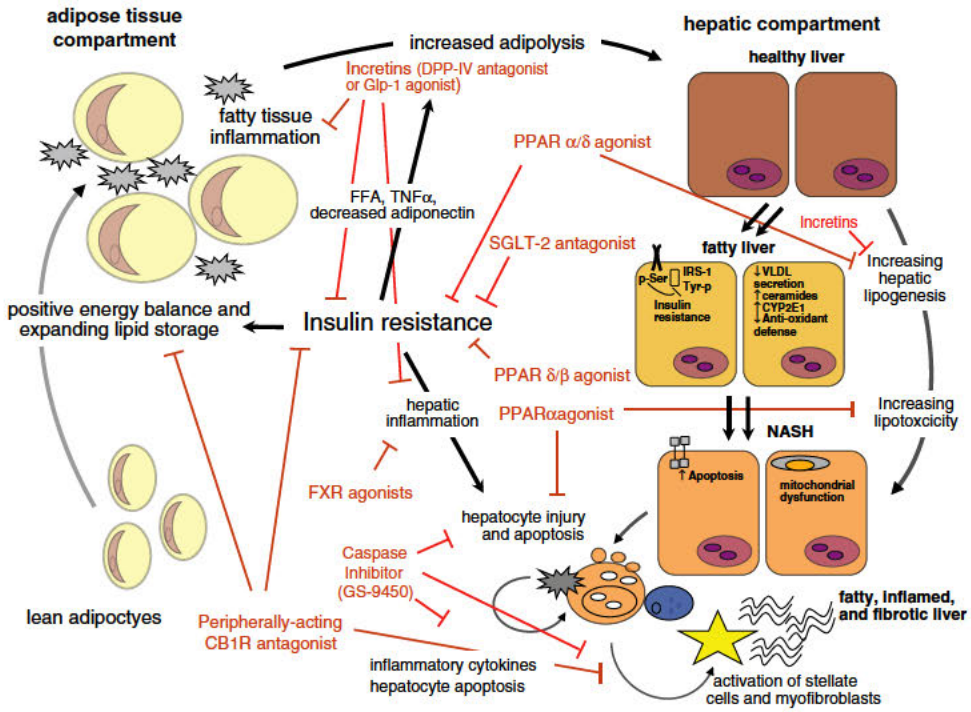
Les principales fonctions de FXR sont[69] :

- inhibition de la gluconéogenèse et la glycogénolyse ;
- amélioration de la sensibilité à l'insuline (dans le muscle squelettique et le tissu adipeux) ;
- inhibition de la lipogenèse via l'inhibition du SREBP1c et l'activation de la beta-oxydation mitochondriale.
- effet anti-inflammatoire via l'inhibition des voies de signalisation NF-kB dépendantes.

L'acide obeticholique est un dérivé synthétique d'acide chenodeoxycholique et un agoniste sélectif des récepteurs FXR. L'efficacité de l'acide obeticholique dans la NASH a été récemment démontré dans un essai de phase II randomisé, contrôlé contre placebo[70].

La **Figure 4** résume le rôle des récepteurs nucléaires dans la physiopathologie de la NASH et identifie les cibles thérapeutiques potentielles.

Figure 4. Le rôle des récepteurs nucléaires dans la physiopathologie de la NASH (d'après Schuppan et al.)[71].



I-4.3. Rôle des facteurs génétiques

Le rôle des facteurs génétiques dans la pathogenèse de la NAFLD résulte de : (1) la démonstration de l'agrégation familiale de la maladie[72] ; (2) les études chez les jumeaux monozygotes[73] ; (3) la variabilité de la prévalence et du phénotype clinique entre les différentes groupes ethniques[18]. A ce jour, il est de plus en plus reconnu que la NAFLD et surtout la progression de la maladie sont le résultat de l'interaction entre les facteurs génétiques, environnementaux et les facteurs d'hôte.

Les études de polymorphisme génétique à l'échelle du génome entier ont identifié plusieurs gènes avec un rôle potentiel dans la pathogenèse de la NAFLD[74].

La PNPLA3 (adiponutrine) est un gène localisée sur le chromosome 22 qui contrôle le flux des AGL vers le foie ainsi que l'expression de certains facteurs de transcription et récepteurs nucléaires qui interviennent dans le métabolisme lipidique. Le résultat est l'accumulation des lipides dans le foie. Les sujets ayant le polymorphisme GG rs738409 sont à risque de développer une maladie plus sévère[47]. Toutefois, à ce jour, il n'existe pas d'études prospectives longitudinales évaluant le rôle du polymorphisme de l'adiponutrine dans la progression des lésions histologiques de la NAFLD. L'hypothèse serait que des facteurs génétiques pourraient expliquer pourquoi certains malades progressent plus vite que d'autres indépendamment des facteurs de risque métabolique.

TM6SF2 (*transmembrane 6 superfamily member 2)* a été aussi associé avec la stéatose[75], la sévérité des lésions histologiques dans la NAFLD et la fibrose avancée[76], la diminution des taux de LDL, de cholestérol total et des triglycérides, l'augmentation des taux de transaminases, l'augmentation du risque de développer un diabète ou des maladies cardiovasculaires[77].

I-4.4. Rôle du microbiote intestinal.

Il a été récemment suggéré qu'en plus de l'axe foie–tissu adipeux, l'axe foie–microbiote intestinal pourrait jouer un rôle majeur dans la physiopathologie de la NAFLD expliquant la progression de la maladie chez certains malades.

La dysbiose, et en particulier la diminution de la diversité microbienne, avec l'augmentation de *Firmicutes* et diminution de *Bacteroides* joue un rôle important au cours de la NAFLD.

Les patients obèses ou ayant une NAFLD ont des enterotypes spécifiques et différents des volontaires sains, malgré des habitudes alimentaires similaires[78].

Le co-hébergement de souris *wild type* ou déficientes dans le récepteur de la leptine avec des souris déficientes dans un composant clé de la formation/activation de l'inflammasome (ASC, Caspase-1, Caspase-11, NLRP3 ou NLRP6) induit l'apparition de la NASH dans plusieurs conditions de régime[79].

D'autres études ont démontré que la transplantation de microbiote de souris insulino-résistantes chez des souris saines s'accompagne d'une hyperglycémie, hyperinsulinémie et stéatose[80].

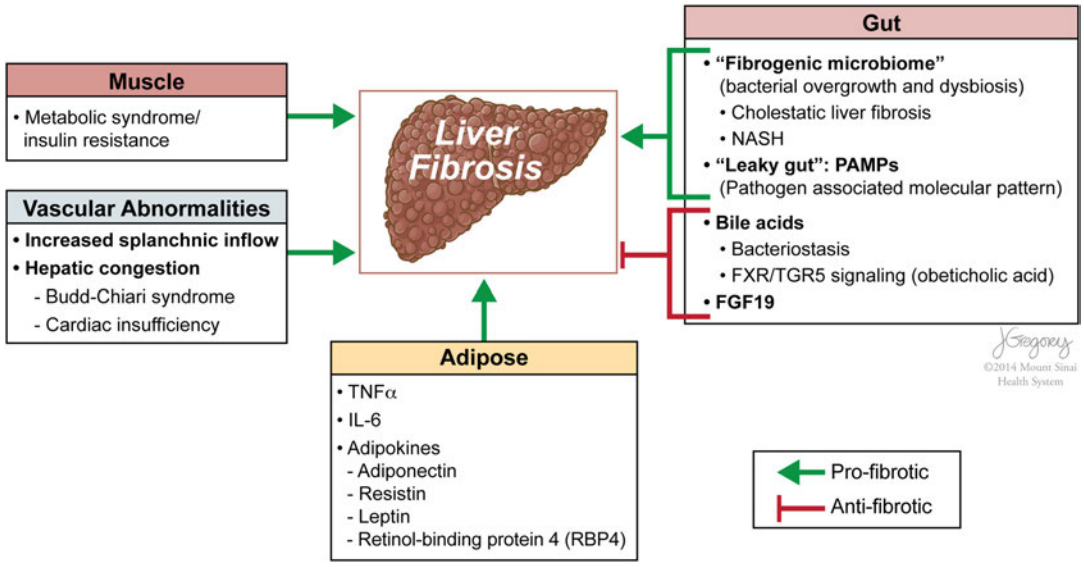
L'augmentation de la perméabilité intestinale entraîne une translocation de LPS (lypoppolysaccharides) dans le flux portal. La liaison du LPS sur son récepteur spécifique le TLR4 et son co-récepteur le CD14 localisés au niveau des cellules de Kupffer déclenche l'activation précoce de la voie NF-kB et la production de cytokines inflammatoires telles que le TNF alpha, IL-6, MCP-1 (Monocyte Chemotactic Protein 1) responsables des dommages hépatiques[81].

Le microbiote intestinal pourrait être utile pour la stratification du risque des patients atteints de NAFLD. Sa manipulation pourra probablement aider à prévenir la progression de la maladie vers ses formes les plus sévères[82, 83].

I-4.5. Mécanismes de progression de la fibrose - applications pratiques

La **Figure 5** résume les principaux mécanismes intervenant dans la progression de la fibrose au cours de la NASH[84]

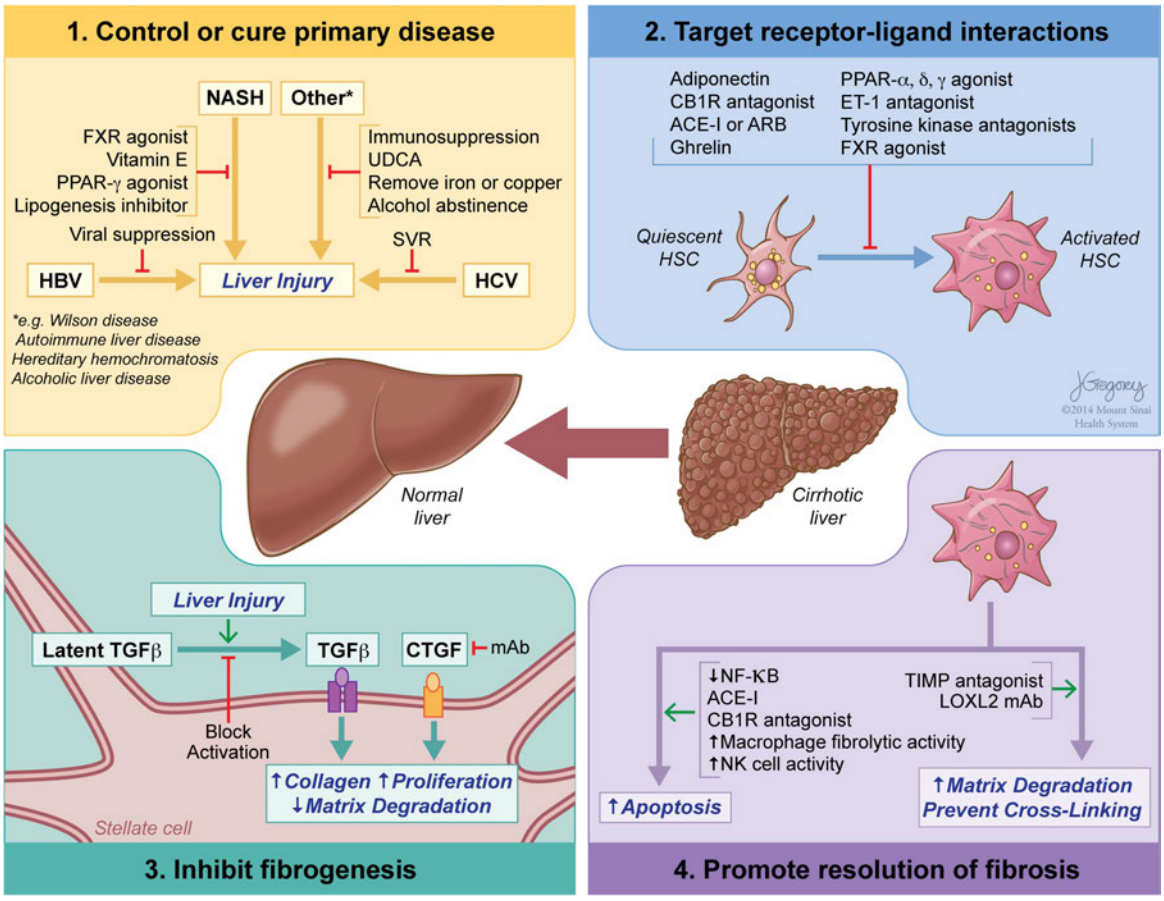
Figure 5. Mécanismes de la fibrogènese (d'après Lee et al.)[84]



L'inflammation chronique associée à l'insulinorésistance et à la dysfonction du tissu adipeux, ainsi que le microbiote intestinal jouent un rôle majeur dans l'activation des cellules étoilées hépatiques et dans la production de matrice extracellulaire. L'identification des stimulis précoces de la fibrogenèse dans le contexte de l'insulinorésistance est essentielle pour une intervention thérapeutique antifibrosante efficace.

La **Figure 6** résume la stratégie thérapeutique dans la NAFLD et suggère les cibles thérapeutiques potentielles émergentes[84].

Figure 6. Nouvelles stratégies et cibles thérapeutiques dans la NAFLD (d'après Lee et al.)[84]



I-5. Diagnostic

Compte-tenu de l'impact de la NAFLD à l'échelle globale en termes de prévalence, des dépenses socio-économiques et de morbi-mortalité, il est essentiel de dépister et diagnostiquer la maladie dans les stades précoces avant que les malades ne développent une fibrose avancée.

A présent, une proportion non négligeables de malades échappent au diagnostic pour des raisons diverses : la méconnaissance de la maladie, son évolution silencieuse souvent avec des transaminases normales, la présence de multiples comorbidités métaboliques et/ou cardiovasculaires qui font que ces patients sont souvent suivis par des endocrinologues et/ou cardiologues mais sont rarement adressés à l'hépatologue.

Généralement, le patient atteint de NAFLD se présente avec des transaminases élevées ; une stéatose à l'échographie concomitante avec un ou plusieurs facteurs de risque métabolique et une hyperferritinémie.

I-5.1. La ponction biopsie hépatique.

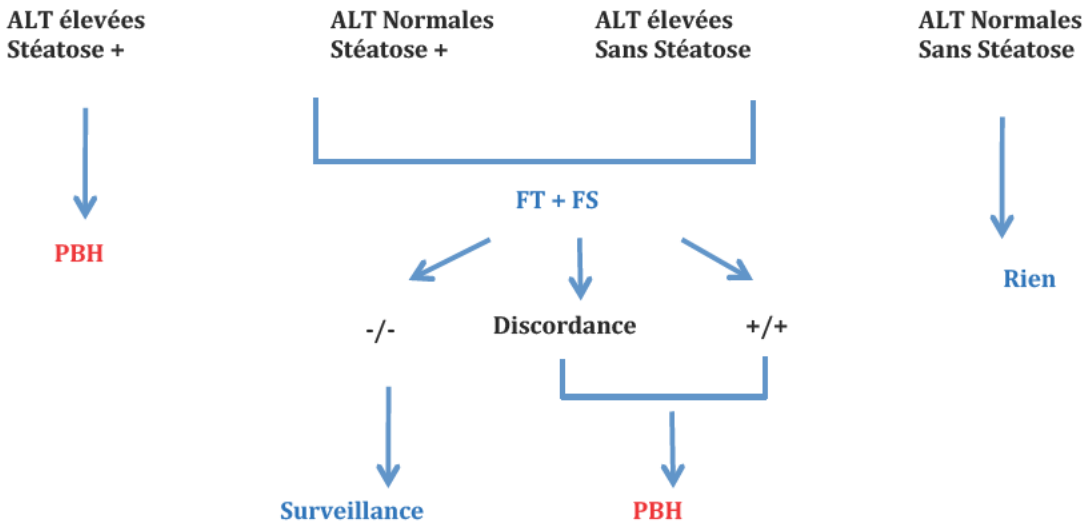
La ponction biopsie hépatique reste à présent le « gold standard » pour le diagnostic de NAFLD.

I-5.1.1. Indication de la biopsie hépatique.

En raison de son caractère invasif, la biopsie hépatique n'est pas indiquée comme méthode de dépistage dans la population générale. L'indication (**Figure 7**) est restreinte aux patients ayant une insulinorésistance (présence de facteurs de risque métabolique, syndrome d'ovaires polykystiques, lipodystrophie, etc) des transaminases élevées et/ou une stéatose à l'échographie ; elle est indiquée en cas de discordance des deux méthodes

non invasives (voir chapitre I-5.2.1. et I-5.2.2) pour estimer la fibrose ; pour les patients avec une insulinorésistance et une autre cause de maladie chronique du foie; et pour les patients ayant subi une chirurgie bariatrique[7, 8].

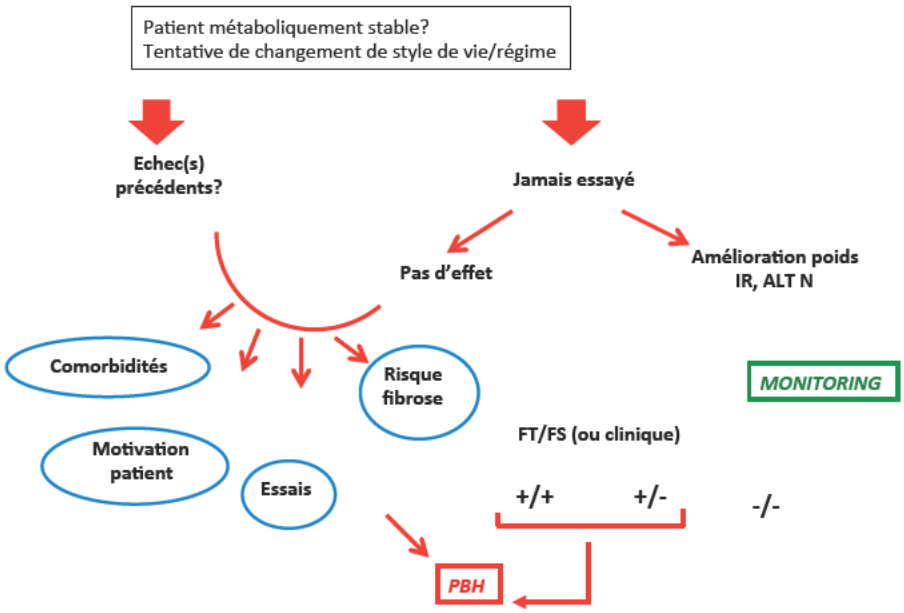
Figure 7. Algorithme décisionnel pour réaliser une ponction biopsie hépatique.



La PBH est indiqué chez les patients avec transaminases élevées et stéatose à l'échographie. Chez les patients avec stéatose à l'échographie et transaminases normales ou les patients avec transaminases élevées sans stéatose à l'échographie, l'indication de réaliser une PBH est restreinte en cas de discordance des deux méthodes non-invasives d'évaluation de la fibrose ou si les deux méthodes indiquent la présence d'une fibrose avancée.

Au décours du suivi la ponction biopsie hépatique peut être indiquée en cas d'échec des mesures hygièno-diététiques, surtout en cas de comorbidités ou facteurs de risque pour la fibrose associées ou pour inclusion dans des essais thérapeutiques (**Figure 8**).

Figure 8. Algorithme décisionnel pour réaliser une ponction biopsie hépatique au décours du suivi.



I-5.1.2. Classification histologique de la NAFLD.

Plusieurs classifications histologiques de la NAFLD ont été proposées. L'évolution de ces classifications au fil du temps indique clairement la nécessité de réaliser une dichotomie claire entre la NAFL (stéatose isolée ou « borderline NASH ») et la NASH (steatohépatite).

La première classification a été élaborée en 1999 par Brunt et al.. Dans cette classification les grades de la maladie (minime, modérée ou sévère) étaient attribués selon une évaluation semi quantitative de la stéatose (0 - pas de stéatose ; 1 – stéatose

<33% ; 2 stéatose 33–66% ; 3 stéatose >66%), de l'inflammation lobulaire (0–3) et de la ballonisation (0–2)[6].

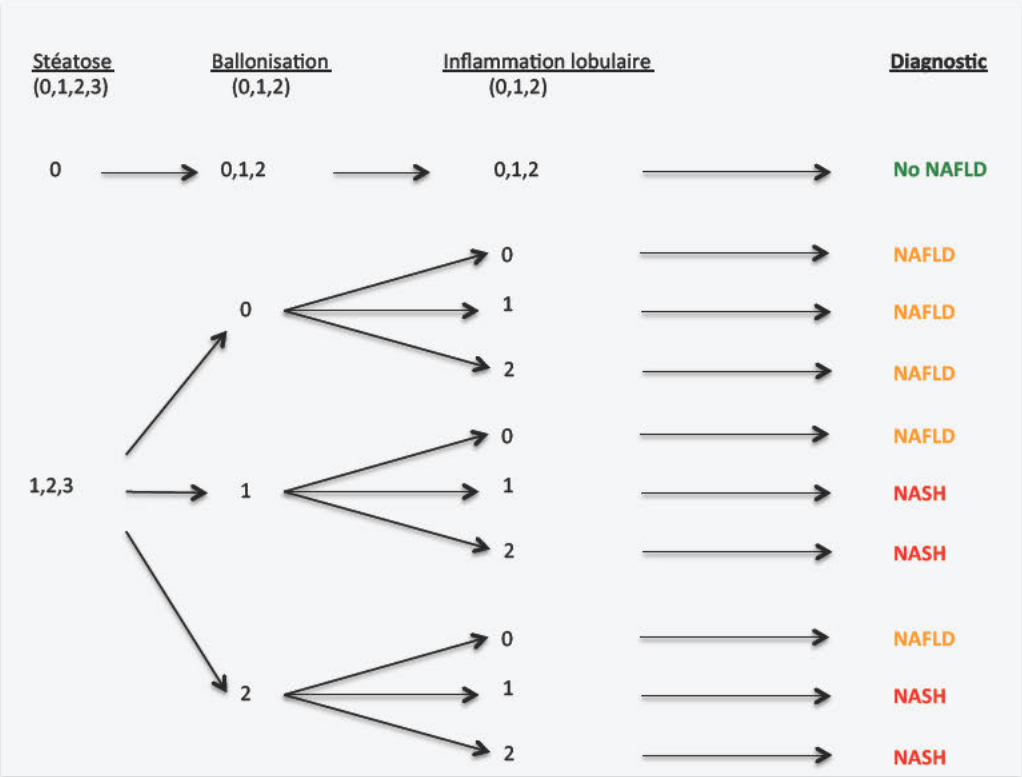
La fibrose est considérée séparément et classée en 4 stades : stade 1 – fibrose périsinusoïdale ; stade 2 – fibrose périsinusoïdale et periportale ; stade 3 – fibrose en pont ; stade 4 – cirrhose.

La même année, 1999, Matteoni et al., propose une classification qui permet une dichotomie entre la stéatose et la NASH reposant sur des données pronostiques : type 1 – stéatose isolée ; type 2 –stéatose avec inflammation lobulaire minime ; type 3 – stéatose et ballonisation hepatocytaire ; type 4 –stéatose, ballonisation hépatocytaire et corps de Mallory ou fibrose. Les types 3 et 4 étaient équivalents avec le diagnostic de NASH.

En 2005, Kleiner et al. développent le score NAS (NAFLD Activity Score) – un score semi quantitatif qui additionne la stéatose, l'inflammation lobulaire et la ballonisation. Le score NAS de 1 ou 2 était considéré comme stéatose isolée ; le score NAS de 3 ou 4 correspondait le plus souvent à « bordeline » NASH ; un score NAS ≥5 correspondait à la NASH[85]. Toutefois, les mêmes auteurs reviennent quelques années plus tard, pour souligner que malgré une bonne corrélation avec le diagnostic NASH/pas NASH, le score NAS, à l'origine, a été conçu pour une évaluation semi-quantitative de la sévérité des lésions histologiques[86]. Effectivement, le score NAS est plus adapté à la quantification des lésions à l'intention d'un usage dans le cadre des interventions thérapeutiques (essais cliniques). La plupart des essais cliniques dans la NAFLD ont comme critère d'efficacité primaire une diminution de 2 points du score NAS sans aggravation de la fibrose.

Enfin, en 2012, Bedossa et al., proposent le score SAF (Steatosis, Activity, Fibrosis), **Figure 9**. La présence d'une stéatose avec lésions concomitantes d'inflammation lobulaire et ballonisation hépatocytaire même minimes définit la NASH[87].

Figure 9. L'algorithme SAF pour le diagnostic de la NASH[87].



I-5.1.3. Limites de la biopsie hépatique.

Les principales limites de la biopsie hépatique dérivent de son caractère invasif et de la variabilité d'échantillonnage et inter-observateur.

Bien que les complications majeures soient rares, les complications mineures, telles que la douleur et le malaise vagal surviennent en 20-30% et respectivement 0.4 – 2% des cas. Les complications majeures (l'hémorragie, la péritonite biliaire, le pneumothorax) surviennent dans 0.5% des cas mais peuvent augmenter à 4.3% des cas si plusieurs passages sont effectués[88].

La variabilité d'échantillonnage est essentiellement due à la petite taille de la biopsie et à l'hétérogénéité de la distribution des lésions histologiques au sein du parenchyme hépatique. Ratziu et al., ont démontré dans une étude que sur 2 biopsies transpariétales effectuées le même jour le taux de discordance pour la présence de la ballonisation est de 18% ; la fibrose était discordante de plus de 2 stades (fibrose en pont sur une biopsie, fibrose minime ou absente sur la 2eme biopsie) dans 35% des cas[89].

La variabilité inter-observateur dérive essentiellement du fait que le diagnostic de NASH et un diagnostic composite qui repose sur l'analyse de plusieurs lésions (stéatose, inflammation, ballonisation). Récemment il a été démontré que la nouvelle classification SAF a une très bonne reproductibilité avec un coefficient de concordance entre plusieurs observateurs de 0.80[90].

I-5.2. Méthodes non invasives de diagnostic.

La plupart des méthodes non invasives développées ont été validées pour le diagnostic de fibrose, mais à présent il n'existe pas une méthode non invasive fiable qui a été validée pour le diagnostic de la NASH.

1-5.2.1. Marqueurs sériques.

Fibrose. Parmi les marqueurs sériques de fibrose, certains ont été validés sur des larges cohortes des patients et brevetés (Fibrotest[91], ELF[92], Fibrometre[93]), d'autres sont des scores calculés sur les données cliniques (NAFLD Fibrosis Score[94], BARD[95], FIB-4[96]). La plupart de ces marqueurs permettent de distinguer la présence d'une fibrose avancée de la fibrose minime ou de l'absence de la fibrose ; certains permettent de distinguer entre les stades adjacents de fibrose[97]. Le Fibrotest a été aussi validé comme facteur pronostique de la mortalité globale et cardiovasculaire[98].

Steatohépatite. Le développement de marqueurs non-invasifs pour le diagnostic de stéatohépatite est difficile en raison des mécanismes multiples et complexes qui interviennent dans la physiopathologie et la progression de la maladie. La valeur diagnostique de la « *caspase generated keratin 18 fragment* » (CK18) [99] n'a pas été confirmée par des études plus récentes [100]. Le NASH Test a été validé sur un nombre limité des patients[101].

Stéatose. Parmi les marqueurs sériques de stéatose, seuls le SteatoTest[102] et le FLI (Fatty Liver Index)[103] ont été validés de manière indépendante. Une étude récente comparant plusieurs marqueurs de stéatose, a montré que l'inflammation et la fibrose sont des facteurs confondants importants pour les performances diagnostiques des marqueurs de stéatose, ce qui limite potentiellement leur applicabilité clinique[104].

I-5.2.2. La mesure de l'élasticité hépatique (FibroScan).

La mesure de l'élasticité du foie chez les malades avec NAFLD permet l'évaluation du stade de fibrose avec un niveau de performance proche de celui retrouvé

dans l'hépatite chronique C[105] et supérieur de celui des scores cliniques (FIB-4, BARD, APRI)[106]. Toutefois, certains aspects doivent être pris en compte dans la mesure de l'élasticité hépatique chez les malades avec NAFLD. La stéatose tout comme l'inflammation augmente modérément l'élasticité hépatique d'environ 1 kPa[107]. L'IMC est corrélé au taux d'échec de la mesure (1% pour un IMC <25 kg/m^2, 17% pour un IMC ≥ 30 kg/m^2 et 42% pour un IMC ≥ 40 kg/m^2)[108]. Chez les patients avec un IMC ≥ 35 kg/m^2, le développement de la sonde XL pour les patients obèses a permis d'obtenir des résultats interprétables dans 65% des cas[109]. Enfin, la variabilité inter-observateur entre 2 mesures de l'élasticité hépatique peut être responsable d'une discordance de 31% pour 1 stade de fibrose et 10% pour ≥ 2 stades[110].

Probablement la combinaison de la mesure de l'élasticité hépatique avec un marqueur sérique permettrait d'augmenter les performances diagnostiques et de restreindre l'indication de la biopsie hépatique.

I-5.2.3. Méthodes d'imagerie.

Des nouvelles techniques d'imagerie par résonance magnétique (IRM) ont été récemment développées pour quantifier la stéatose et la fibrose. Récemment il a été démontré que l'elastographie par résonance magnétique (2D-IRM) a une très bonne performance diagnostique pour la fibrose avancée, indépendamment de la quantité de stéatose[111] et avec une valeur prédictive nettement supérieure aux scores cliniques (APRI, BARD, FIB-4 et NAFLD Fibrosis Score) de fibrose[112].

Des nouvelles techniques de spectroscopie par résonance magnétique et de résonance magnétique par diffusion de protons ont été développées pour le diagnostic quantitatif de la stéatose. Ces techniques ont une performance diagnostique similaire

voir meilleure que la biopsie hépatique pour la détection de la stéatose de < 5%. La résonance magnétique par diffusion de protons a l'avantage de quantifier la stéatose dans chaque segment hépatique et diminue ainsi le risque de variabilité d'échantillonnage propre à la biopsie hépatique[113].

Cependant, l'utilisation en pratique courante de ces techniques est limitée par leur coût et leur disponibilité limitée pour l'instant à des centres de recherche.

Chapitre II. L'histoire naturelle de la NAFLD.

II-1 Fibrose

L'histoire naturelle de la NAFLD a été décrite à partir d'études transversales menées dans la population générale ou d'études avec biopsies hépatiques séquentielles.

Dans les séries rétrospectives issues de centres spécialisés, une fibrose hépatique significative (fibrose en pont) est retrouvée dans 25-33% des cas de stéatohépatite lors du diagnostic dont 10-15% de cirrhoses constituées[114].

Pendant longtemps, la NAFLD a été considérée comme une maladie avec une progression lente de la fibrose chez un nombre limité des malades. Dans les maladies chroniques du foie, la fibrose est le résultat d'un équilibre dynamique entre les processus de fibrogènese et de fibrinolyse. Comparé à l'hépatite C, dans les stades précoces, les malades avec NAFLD ont une quantité moins importante de fibrose mesurée par techniques de morphométrie. En revanche, dans les stades avancés, la quantité de fibrose dans la NAFLD est équivalente avec la quantité de fibrose chez les malades avec l'hépatite C[115]. La fibrose périsinusoïdale, présente dans la NAFLD mais absente dans l'hépatite C ainsi que les mécanismes différents intervenants dans la fibrose viro-induite et la fibrose dans les maladies métaboliques, peuvent expliquer l'augmentation de la quantité de fibrose dans la NAFLD dans les stades plus avancés de la maladie.

La prévalence réelle de la cirrhose due à la NAFLD est probablement sous estimée. Le diagnostic de NAFLD au stade de cirrhose est souvent difficile car les lésions histologiques typiques ne sont plus présentes. La recherche d'éléments présents ou passés du syndrome métabolique ou la documentation d'une stéatose non alcoolique et non virale sur une échographie ancienne permettent souvent de redresser le diagnostic.

Particulièrement, la stéatose diminue avec la progression de la fibrose. Ce phénomène dont le mécanisme n'est pas clair, est typique pour la NAFLD est n'est pas décrit dans l'histoire naturelle d'autres maladies chroniques du foie. Une des hypothèses possibles serait une diminution de l'exposition des hépatocytes aux AGL en raison des shunts portaux existantes dans la cirrhose[115].

La NAFLD est une cause fréquente de cirrhose cryptogénétique retrouvée dans 30 à 75% des cas[116]. Des séries européennes et américaines des patients transplantés hépatique identifient la NASH chez 44% des patients avec un diagnostic initial de cirrhose cryptogénétique[117,118]. Ces résultats sont confirmés par l'analyse du registre américain de transplantation qui montre que l'augmentation des cas de cirrhose cryptogénétique est parallèle à l'augmentation de l'IMC (10% des cas de cirrhose cryptogénétique chez les patients avec un IMC < 25 kg/m^2 vs. 21% chez les patients avec un IMC ≥ 30 kg/m^2)[119].

II-1.1. Etudes avec biopsies répétées

L'histoire naturelle de la NAFLD reste encore mal connue. La NAFLD est une maladie complexe issue de l'interaction de multiples facteurs et voies pathogéniques dont le résultat conduit à une grande hétérogénéité des phénotypes cliniques. Particulièrement le profil de malades à risque de progression ainsi que les facteurs responsables de l'aggravation des lésions histologiques sont loin d'être définis. Plusieurs études observationnelles rétrospectives avec des biopsies hépatiques répétées issues de centres tertiaires de référence ont analysé la progression des lésions histologiques dans la NAFLD (**Tableau 4**)[120-132].

Tableau 4. Progression de la fibrose dans les études avec biopsies hépatiques répétées.

Auteur, année	Pays	Durée de suivi (ans, moyenne ; rang)	Nombre des patients avec 2 biopsies	Classification histologique	Evolution de la fibrose		
					Régression	Stable	Progression
Powell, 1990	Australie	7.3 (2,5 21.5)	13 ; (NAFL = 0, NASH = 13)	NR	3 (23%)	6 (46%)	4 (31%)
Teli, 1995	Angleterre	11.6 (7.6 -16)	12 ; (NAFL = 12, NASH = 0)	NR	0	11 (92%)	1 (8%)
Ratziu, 2000	France	NR (1.5 – 15)	14 ; (NAFL = 10, NASH = 4)	Metavir	4 (29%)	8 (57%)	2 (14%)
Evans, 2002	Angleterre	8.2 (5.5 – 11.9)	7 ; (NAFL = 0, NASH = 7)	Brunt	0	3 (43%)	4 (57%)
Harrison, 2003	Etats Unis	5.7 (1.4 – 15.7)	22 ; (NAFL = 0, NASH = 22)	Brunt	4 (18%)	11 (50%)	7 (32%)
Fassio, 2004	Argentine	5.3 (3 – 14.3)	22 ; (NAFL = 0, NASH = 22)	Brunt, Ishak	4 (18%)	11 (50%)	7 (32%)
Adams, 2005	Etats Unis	3.2 (0.7 – 21)	103 ; (NAFL = 7, NASH = 96)	Brunt	30 (29%)	35 (34%)	38 (37%)
Hui, 2005	Hong Kong	6.1 (3.8 – 8)	17 ; (NAFL = 3, NASH = 14)	Brunt	0	8 (47%)	9 (53%)
Ekstedt, 2006	Suède	13.8 (10.3 – 16.3)	70 ; (NAFL = 36, NASH = 34)	Brunt	11 (16%)	30 (43%)	29 (41%)
Hamaguchi, 2010	Japon	2.4 (1 – 8.5)	39 ; (NAFL = 22, NASH = 17)	Brunt	12 (31%)	16 (41%)	11 (28%)
Wong, 2010	Hong Kong	3 (NR)	52 ; (NAFL = 35, NASH = 17)	Kleiner et Brunt	13 (25%)	25 (48%)	14 (27%)
Pais, 2013	France	3.7 (NR)	70 ; (NAFL = 25, NASH = 45)	Kleiner et Brunt	20 (29%)	30 (42%)	20 29%)
Kleiner, 2013	Etats Unis	4.4 (1 – 17.3)	359 ; (NAFL, NASH, NR)	Kleiner, Brunt	102 (28%)	128 (36%)	128 (36%)
McPherson, 2015	Angleterre	6.6 (1.3 – 22.6)	108 ; (NAFL = 27, NASH = 81)	Brunt	20 (18%)	43 (40%)	45 (42%)

Les principales questions soulevées par ces études sont :

- Est-ce que les malades avec stéatose isolée peuvent progresser vers la NASH et la fibrose ?
- Quels sont les facteurs de risque pour l'aggravation des lésions histologiques ?
- Quelle est la vitesse de progression de la fibrose et quels sont les facteurs individuels qui la déterminent ?

II-1.1.1. Evolution des patients avec une stéatose pure.

Le dogme classique stipule que les malades avec stéatose isolée ont une forme bénigne de la maladie qui ne progresse pas.

Toutefois, des cas isolés de progression de la maladie ont été rapportés chez ces malades. Harrison et al., ont rapporté deux cas de stéatose isolée qui ont développé une inflammation et une fibrose minime (stade 1) dans un intervalle de 4.5 et 14.5 ans[124]. Adams et al., ont rapporté 2 malades avec stéatose pure et 4 malades avec stéatose et inflammation minime qui ont progressé vers la NASH[126]. Dans l'étude de Hui et al., 1 malade avec stéatose pure a progressé vers la NASH malgré une perte de poids significative au bout de 4.2 ans de suivi[127]. Parmi 36 malades avec stéatose et inflammation minime lobulaire ou portale, dans l'étude de Ekstedt et al., 17 ont développé une fibrose pendant la période de suivie (13.8 ans en moyenne)[128]. Enfin, dans l'étude de Wong et al., parmi 13 patients avec stéatose pure, 3 ont développé une NASH[130].

Ces résultats suggèrent que la progression de la stéatose pure vers la NASH est possible mais rare et limitée à des cas isolés. Toutefois, ces résultats doivent être interprétés avec prudence, dans la plupart des cas la progression est minime (un grade d'inflammation ou ballonisation ou de fibrose) qui peut être attribué à l'erreur de l'échantillonnage de la biopsie hépatique. Les facteurs associés à la transition vers la NASH ne sont pas renseignés dans aucune de ces études.

Plus récemment, McPherson et al.[132], décrivent l 'évolution de 27 patients avec NAFL pendant une période de suivie moyen de 6.6 ans. Parmi ces patients, 12 (44%) ont développé une NASH (4/7 avec stéatose pure et 8/10 avec stéatose et inflammation minime), la résolution de la NASH est survenue uniquement dans 7.5 % des cas.

L'ensemble de ces résultats nous apprennent que : (1) La présence d'une inflammation même minime augmente le risque de progression et de développer une NASH. Par conséquent, ces malades doivent être suivis régulièrement et bénéficier de mesures thérapeutiques spécifiques. (2) Une fois la NASH constituée, la résolution spontanée de la maladie en absence d'interventions thérapeutiques spécifiques est très rare.

II-1.1.2. Progression de la fibrose.

La progression de la fibrose dans ces études varie entre 8% et 53% et la régression entre 16% et 31% des cas. Des études décrivent la progression de la fibrose malgré une régression de la stéatose et/ou de l'inflammation[124], malgré une diminution des transaminases[126] ou malgré une perte du poids[125].

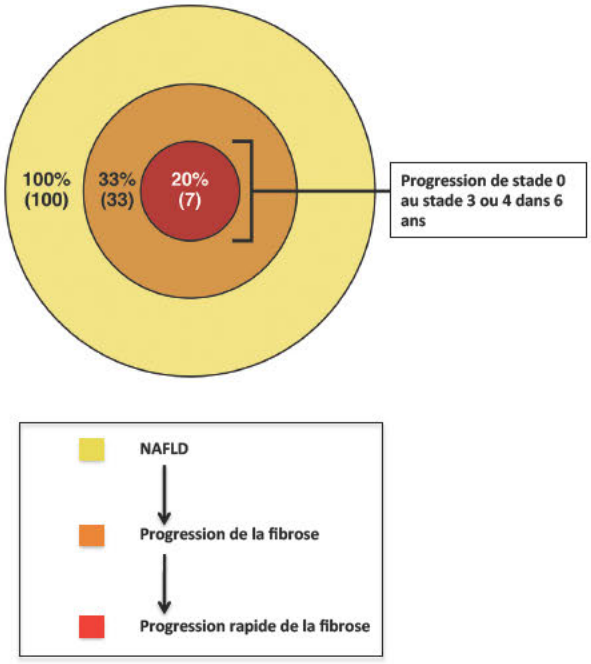
Récemment, 11 de ces études ont été incluses dans une méta-analyse : 411 patients avec NAFLD dont 150 avec stéatose isolée et 261 avec NASH[133]. Pendant une période de

suivi de 2145.5 personnes-année, la progression de la fibrose est survenue chez 33.6% des malades et la régression chez 22.3%. Le taux annuel de progression de la fibrose était de 0.07 stade par an chez les patients avec stéatose sans fibrose et de 0.14 stade par an chez les patients avec NASH sans fibrose au début du suivi. Cela correspond à une progression d'un stade en 14 ans pour les patients avec stéatose et d'un stade en 7 ans pour les patients avec NASH. La proportion des patients avec une progression significative de la fibrose (de stade 0 au stade 3 ou 4) était similaire pour les patients avec stéatose et NASH (17% et respectivement 18%). Contrairement à une étude précedente[134] qui retrouvait une corrélation positive entre la présence de lésions de nécroinflammation et la progression de la fibrose, dans cette méta-analyse aucune corrélation n'était retrouvée. Toutefois, dans l'étude de McPherson et al., ainsi que dans notre étude (voir Chapitre V) et deux autres études antérieurs, la fibrose progressait plus chez les patients avec stéatose et minimes lésions d'inflammation lobulaire [126, 128, 132, 135]. Une autre étude analysant 728 patients avec NAFLD provenant du registre américain NASH CRN suggère que l'inflammation portale est significativement associée avec la sévérité des lésions histologiques (i.e. NASH et fibrose avancée) et donc pourrait être un facteur de risque de progression de la maladie[136]. Chez les patients sans fibrose ou avec fibrose minime (stade 0 et 1), le taux annuel de progression de la fibrose est similaire pour la stéatose et pour la stéatohépatite, la présence de fibrose sur la biopsie initiale n'étant pas corrélée sa progression [133].

Globalement, les résultats de cette méta-analyse suggèrent qu'environ un tiers de patients avec NAFLD sont à risque de progression fibrosante et que parmi ces derniers, 20% sont à risque de progresser du stade 0 au stade 3 ou 4 sur une durée moyenne de 6 ans environ (**Figure 10**).

Compte-tenu de la prévalence actuelle de la stéatose dans la population générale et particulièrement dans la population pédiatrique, la projection des ces estimations conduit à des chiffres alarmants concernant le nombre de patients potentiellement à risque de développer une fibrose avancée dans les 20–30 ans à venir.

Figure 10. Progression de la fibrose. Parmi 100 patients avec NAFLD, 33 sont à risque d'avoir une progression de la fibrose. Parmi ces derniers, 7 (20%) sont à risque de progresser de 3 stades ou plus dans un intervalle moyenne de 6 ans. (Adaptée selon Harrison, 2015)[137].



II-1.2. Facteurs prédictifs pour la progression de la fibrose

Dans la plupart des études longitudinales avec biopsies répétées, la progression de la fibrose était associée à une persistance ou une aggravation des facteurs de risque

métabolique. Ce dernier point a été confirmé par une large série provenant du registre américain NASH CRN. Parmi 270 malades avec NAFLD sans fibrose avancée (stades 0 à 2), les facteurs associés à la progression de la fibrose (16% dans un intervalle moyen de 4.4 ans) étaient le diabète de type 2, le syndrome métabolique et l'index HOMA IR[138].

L'ancienneté de la maladie et la durée de l'exposition aux différents facteurs de risque pourraient jouer un rôle important.

L'obésité est un facteur de risque indépendant connu pour la progression de la fibrose et le développement de la cirrhose, elle est présente dans 55% des patients présentant une cirrhose cryptogénétique[139]. Le risque relatif de développer une cirrhose augmente de 28% pour une augmentation de 5 unités de l'IMC[140].

Parfois l'obésité et l'alcool ont un effet additif sur le risque de développer une fibrose avancée ou une cirrhose. Cependant, le seuil de la consommation d'alcool admis chez les malades avec NAFLD n'est pas bien établi. Une étude récente montre qu'une consommation modérée d'alcool (<2 verres par jour) pourrait avoir un effet protecteur pour la NASH[141]. Une des explications pourrait être l'amélioration des facteurs de risque métabolique via l'effet protecteur de l'alcool sur l'insulinorésistance[142]. D'autres études ont montré que la consommation modérée de l'alcool diminue de 38% le risque cardiovasculaire[143] ainsi que la mortalité globale[144]. Toutefois, la consommation d'alcool même modérée n'est pas actuellement recommandée par les sociétés savantes Américaine et Européenne chez les malades avec NAFLD[7, 8].

Le diabète de type 2 est un facteur de risque indépendant associé avec la sévérité de la fibrose même chez les patients avec des transaminases normales[29]. Au niveau de la population générale, le diabète de type 2 est associé avec une incidence annuelle de maladie sévère du foie de 8.19 per 10 000 personnes-année[145]. Le risque relatif de

développer une insuffisance hépatocellulaire est de 2.3 per 10 000 personnes-année chez les patients diabétiques[146].

D'autres facteurs vraisemblablement associés avec des formes plus sévères de NAFLD (facteurs génétiques, le microbiote, le syndrome d'apnée de sommeil, etc) ont été analysés dans des études transversales. Le rôle des facteurs génétiques et du microbiote dans la sévérité des lésions histologiques dans la NAFLD a été discuté dans le chapitre I de cette thèse (I-4.4 et I-4.5).

Le syndrome d'apnée de sommeil émerge comme un nouveau facteur de risque associé avec la sévérité des lésions hépatiques dans la NAFLD[147, 148]. En effet, le syndrome d'apnée de sommeil induit une hypoxie chronique qui aggrave l'insulinorésistance et le diabète type 2[149].

En conclusion, les données de la littérature, suggèrent que les patients ayant une stéatose sévère, des lésions même minimes d'inflammation lobulaire, de nécrose hepatocytaire ou ceux avec persistance ou aggravation des facteurs de risque métabolique sont à risque de progression de la maladie. Ces patients doivent bénéficier d'une prise en charge renforcée par des mesures thérapeutiques spécifiques. Toutefois, des études prospectives longitudinales avec un suivi à long-terme sont nécessaires afin de confirmer ces résultats issus d'études transversales ou longitudinales rétrospectives.

II-1.3. Différences et similitudes avec la cirrhose d'autres étiologies

Chez les patients atteints de NAFLD la cirrhose survient à un âge plus tardif que chez ceux infectés par le VHC. [150]. Une des hypothèses pouvant expliquer ces différences

est le retard diagnostique due à la méconnaissance de la NAFLD et l'absence de méthodes non invasives fiables de dépistage. Une fois la cirrhose constituée, la survie des patients avec cirrhose décompensée (Child B ou C) est similaire chez les patients avec NAFLD ou VHC[150].

Une étude américaine montre que dans la cirrhose Child A, les patients avec NAFLD ont un meilleur pronostic que les patients infectés par le VHC en termes de survie ou de risque de décompensation. En revanche, dans les stades Child B et C le risque de décompensation (rupture des varices oesophagiennes, ascite, encéphalopathie) et de décès, était similaire chez les patients avec NAFLD et l'hépatite C. La première cause de décès dans les deux groupes était le risque d'infections suivie par le décès de causes cardiovasculaires dans le groupe NAFLD mais pas dans le groupe VHC[151].

Plus récemment, une étude collaborative internationale (Etats Unis, Australie, Italie et Angleterre) a comparé 247 patients avec NAFLD et fibrose avancée (stade 3) ou cirrhose compensée (Child A) avec 264 patients avec cirrhose VHC Child A. Alors que le risque de décompensation de la cirrhose et de morbidité liée au foie était plus élevé chez les patients avec l'hépatite C, le risque de mortalité globale et cardiovasculaire était similaire pour les deux cohortes[152]. Cependant, ces résultats doivent être interprétés avec prudence car dans le groupe NAFLD une partie des patients n'était pas cirrhotiques. D'autre part, les patients inclus dans le groupe VHC avaient potentiellement une maladie plus sévère car il s'agissait de patients non traités (trop graves pour être traités ?) ou des non-répondeurs aux traitements.

Il est possible que dans les années à venir la cirrhose due à la NAFLD dépasse en termes de gravité, de mortalité et de morbidité globale ou spécifique la cirrhose liée au virus de l'hépatite C pour les raisons suivantes : (1) les nouveaux antiviraux directs permettront la guérison d'une grande partie des patients infectés par le VHC induisant

une amélioration de leur pronostic à moyen et long-terme. (2) la prévalence de la NAFLD et des maladies sévères du foie dues à la NAFLD est susceptible d'augmenter. Ces patients sont plus âgés et ont de multiples comorbidités qui aggravent leur pronostic et limitent l'accès au traitement.

II-2. NAFLD et transplantation hépatique

La NAFLD est la 2éme indication de transplantation hépatique aux Etats Unis. Pendant les 10 dernières années, la prévalence de la NAFLD comme indication pour la transplantation hépatique a augmenté de 170% alors que dans la même période, la prévalence de la maladie alcoolique du foie et de l'hépatite chronique C comme indication de transplantation hépatique a augmenté de 45% et 14% respectivement[153].

La NAFLD est la 2éme cause de transplantation hépatique pour carcinome hépatocellulaire. La proportion des cas de carcinome hépatocellulaire développé sur cirrhose NASH ou cryptogénétique a augmenté de 8.2% en 2002 à 13.5% en 2012[154].

Les patients avec NAFLD ont souvent une atteinte rénale, parfois sévère, qui explique l'augmentation des cas de double transplantation foie–rein (8.2% en 2002 a 22% en 2011)[155].

Enfin, les patients avec NAFLD ont un accès limité à la transplantation et sont plus à risque de décéder ou d'être retirés de la liste de greffe en raison de leur âge avancée, de l'obésité et des multiples comorbidités associées[156].

II-3. Survie

Les données de survie chez les patients atteints d'une stéatose proviennent de larges études de cohorte dans la population générale utilisant des marqueurs indirects

pour la NAFLD (transaminases, GGT, stéatose à l'écographie ou l'agrégation des facteurs de risque métabolique) ou d'études réalisées dans centres tertiaires de référence chez de patients avec NAFLD bien phénotypé.

II-3.1. Population générale

Il a été démontré que dans la population générale l'élévation de la GGT est associée avec une augmentation du risque de mortalité globale ou liée au foie[157]. Ces résultats ont été confirmés par une autre étude qui montre que la présence concomitante de GGT élevée avec stéatose à l'échographie est associée avec une augmentation significative de la mortalité globale et particulièrement avec un risque relatif de décès de causes cardiovasculaires 6 fois plus élevé[158].

Une augmentation de l'IMC de 5 kg/m^2 est associée avec une augmentation de 30% de la mortalité globale, 40% de la mortalité cardiovasculaire et 60–120% de la mortalité liée au diabète et au foie. L'espérance de vie est diminuée en moyenne de 2 à 4 ans chez les personnes avec un IMC de 30–35 kg/m^2 et de 8 à 10 ans chez les personnes avec une IMC de 40– 5 kg/m^2 (magnitude de l'effet similaire avec le tabac)[159].

Le diabète de type 2 est un facteur indépendant de décès responsable d'une augmentation significative de la mortalité globale, cardiovasculaire ou liée au foie[160].

Les composantes du syndrome métabolique sont responsables d'une augmentation significative de la mortalité globale et spécifique chez les patients avec NAFLD mais aussi chez les patients avec d'autres causes de maladie chronique du foie[161].

Les patients avec NAFLD ont une mortalité globale et spécifique à 10 ans augmentée de 55% par rapport à la population générale après appariement selon l'âge et le sexe. Les maladies cardiovasculaires et le cancer sont les premières causes de décès

chez les patients avec NAFLD. La mortalité hépatique est la 3éme cause de décès chez les patients avec NAFLD et la 1ere dans la population générale[162, 163].

II-3.2 Centres tertiaires de référence

A long terme (période de suivi de 28 ans), les patients avec NAFLD prouvée histologiquement ont une augmentation significative de la mortalité globale de 69% par rapport à la population générale après appariement selon l'âge et le sexe[164].

La stéatose isolée n 'augmente pas la mortalité globale, alors que la NASH augmente la mortalité de 35 à 85% par rapport à la population générale[128, 164]. Parmi les patients avec une cirrhose pour lesquels un suivi était disponible à 10 et 21 ans, la proportion de décès était de 19% et de 89% respectivement, le décès était lié au foie dans 14.5 et 44% des cas[151, 164]. Dans l'ordre, dans la plupart des ces études les principales causes de décès étaient les maladies cardiovasculaires, le cancer et les décès liés au foie. Les principales études avec une durée de suivi de plus de 5 ans qui ont analysé la mortalité globale et spécifique chez les patients avec NAFLD sont résumées dans le **Tableau 5** [121, 123, 128, 163-166] (adapté selon Angulo et al [167]).

Tableau 5. Etudes avec une durée de suivi de plus de 5 ans qui ont analysé la mortalité globale et spécifique chez les patients avec NAFLD prouvée histologique (d'après Angulo et al.)

Auteur	Diagnostic	N	Durée de suivi (ans)	Prevalence de la cirrhose (n, %)	Mortalité liée au foie (n, %)	Mortalité globale (n, %)
Adams	NAFLD	420	7.6	21 (5%)	7 (1.7%)	53 (12.6%)
Ekstedt	NAFLD	120	13.7	10 (7.8%)	2 (1.6%)	26 (20.2%)
Rafiq	NAFLD	131	18.5	NR	12 (9.2%)	78 (59.5%)
Soderberg	NAFLD	118	21	9 (7.6%)	9 (7.6%)	47 (39.8%)
	Total	**798**	**15.2**	**40 (6%)**	**30 (3.8%)**	**204 (25.6%)**
Teli	Stéatose pure	40	9.6	0	0	14 (35%)
Ekstedt	Stéatose pure	58	13.7	0	0	7 (12.1%)
Dam Larsen	Stéatose pure	170	20.7	2 (1.2%)	1 (0.6%)	48 (28.2%)
Rafiq	Stéatose pure	74	18.5	NR	2 (2.7%)	42 (56.8%0
	Total	**342**	**15.6**	**2 (0.7%)**	**3 (0.9%)**	**111 (32.5%)**
Evans	NASH	26	8.7	1 (4%)	0	4 (15%)
Ekstedt	NASH	71	13.7	10 (14.1%)	2 (2.8%)	19 (26.8%)
Rafiq	NASH	57	18.5	NR	10 (17.5%)	36 (63.2%)
Soderberg	NASH	51	21	5 (9.8%)	3 (5.9%)	24 (47.1%)
	Total	**205**	**15.5**	**16 (10.8%)**	**15 (7.3%)**	**83 (40.5%)**

II-3.3. Fibrose et survie

En raison de la prévalence élevée de la NAFLD dans la population générale et du pronostic variable d'un individu à l'autre, c'est important d'établir la valeur pronostique des lésions histologiques de la NAFLD, notamment la stéatose, la steatohépatite et la fibrose. Cela permettrait de mieux cibler les patients à risque qui nécessitent une surveillance rapprochée et une prise en charge thérapeutique spécifique.

Dans une étude récente, Ekstedt et al., démontrent que la fibrose avancée (i.e. stades 3 et 4) est associée avec une augmentation significative de la mortalité globale et spécifique, notamment cardiovasculaire et hépatique, alors que la stéatose ou la NASH (présumées par un score NAS de 0 – 4 et respectivement 5-8) sans fibrose avancée (≤ 2) n'augmentent pas la mortalité globale ou cardiovasculaire[168]. Toutefois, ces résultats doivent être interprétés avec prudence. Premièrement, le nombre des patients avec fibrose avancée était limité (27/229 patients). Deuxièmement, le score NAS n'est pas un score diagnostique[169]. Donc l'étude peut uniquement conclure sur l'absence de la corrélation entre le score NAS et la mortalité globale ou spécifique mais ne peut pas conclure sur la corrélation entre la NASH et la mortalité globale ou spécifique.

Une deuxième étude (l'étude PREHLIN) a analysé la corrélation entre les lésions histologiques et la mortalité globale et spécifique chez 619 patients avec NAFLD suivis pendant 12.6 ans. Parmi les lésions histologiques, uniquement la fibrose était un facteur prédictif indépendant pour la mortalité globale et spécifique. Cette observation a été maintenue quand les patients avec fibrose avancée (stade 3 et 4) ont été exclus de l'analyse[170].

Ces résultats sont d'une importance considérable car ils identifient les patients ayant une fibrose (quelque soit le stade) comme étant le plus à risque pour développer

des complications à long-terme. Cependant, dans la pratique clinique et la prise en charge quotidienne des patients, il faut tenir compte du fait que les malades avec NASH sont ceux à risque de développer ultérieurement une progression de la fibrose. Par conséquent, même en l'absence de la fibrose, les malades avec NASH doivent être considérés comme des malades à risque et bénéficier d'une prise en charge et d'un traitement adapté.

Chapitre III. La NAFLD et le carcinome hépatocellulaire

Le carcinome hépatocellulaire (CHC) est la 5-éme cause de cancer au niveau mondial et la 3-éme cause de décès liée au cancer. L'incidence du CHC varie beaucoup selon la région géographique. Dans les pays Asiatiques l'incidence de CHC est de 31.9/100 000 personnes chez les hommes et 10.2/100 000 personnes chez les femmes. Aux Etats-Unis l'incidence du CHC est de 9.3/100 000 personnes chez les hommes et 2.7/100 000 chez les femmes. En Europe de l'Ouest incidence de CHC est de 8/100 000 chez les hommes et 2.2/100 000 chez les femmes. Selon des estimations récentes de GLOBOCAN le nombre des nouveaux cas de CHC en 2012 était de 782 500 et le nombre des décès liés au CHC de 745 500[171].

Historiquement, les causes les plus fréquentes de CHC étaient les hépatites virales B et C et la maladie alcoolique du foie. Toutefois, compte tenu du pic d'incidence de l'infection par le VHC dans les années 1960 – 1980 ainsi que des avancées récentes dans le traitement qui permettent actuellement la guérison de l'hépatite C, il est probable que le nombre des cas de CHC dus à l'hépatite C ne va pas augmenter dans les années à venir[172, 173]. Dans une analyse récente de la base de données MEDICARE aux Etats Unis le risque relatif de CHC dû à l'hépatite C était de 39.9 alors que le risque relatif de CHC dû à l'obésité et au diabète de type 2 était de 2.47. Cependant, en raison de leurs prévalence élevée au Etats Unis, le diabète de type 2 et l'obésité étaient responsables de plus des cas de CHC que l'hépatite C. Dans la population générale, la fraction étiologique du risque de CHC attribuable au diabète et à l'obésité était de 36.6% alors que cellec-ci n'était que de 23.5% pour l'hépatite C et 22.4% pour l'hépatite B [174].

En raison de la prévalence croissante de l'obésité et du diabète et de leurs liens étroits avec la NAFLD ainsi que des avancées thérapeutiques permettant le contrôle des

autres causes des maladies chroniques du foie, il est possible que la NAFLD devienne la cause la plus fréquente de CHC dans les années à venir.

III – 1. Obésité et carcinome hépatocellulaire

Le CHC est le cancer dont l'occurrence et la progression sont le plus fort liées à l'obésité. A titre d'exemple, dans un large étude prospective américaine conduite chez plus de 900 000 participants suivis pour une période de 16 ans, le risque relatif de décès lié au CHC chez les personnes obeses (IMC > 35 kg/m^2) était de 4.52 chez les hommes et 1.68 chez les femmes. La proportion des décès dues au cancers attribuables au surpoids et l'obésité était de 4.2 – 14.2 % chez les hommes et 14.3% - 19.8% chez les femmes[175]. Dans une étude européenne, le risque de CHC était 3.5 fois plus élevé chez les sujets obèses avec une augmentation supplémentaire du risque chez les sujets obèses et diabétiques (OR = 11.8, 95% CI 2.7 – 51.9)[176].

L'association entre l'obésité et l'augmentation du risque de CHC a été aussi confirmée chez les sujets asiatiques. Au Japon, le risque de CHC était 2 fois plus élevé chez les patients en surpoids avec d'autres facteurs de risque associés (HR = 1.99, 95% CI 1.11 – 3.58)[177]. Ces résultats ont été confirmés par une large étude de cohorte en Corée. Cette étude montre une association positive entre l'obésité et le risque de CHC même après ajustement pour d'autres facteurs de risque, notamment le virus de l'hépatite B dont la prévalence est élevée dans cette région[178].

Dans une méta-analyse récente incluant 11 études de cohorte, le risque de CHC était augmenté de 17% chez les patients en surpoids et de 90% chez les obèses. 28% des cas de CHC étaient attribuables à un excès pondéral. L'excès du risque relatif de CHC dû au surpoids et à l'obésité était de 117% et 189% respectivement[179]. Toutefois, cette méta- analyse n'a pas ajusté le risque de CHC pour les facteurs confondants tels que le

virus de l'hépatite B, C ou la consommation excessive d'alcool. Ces résultats ont été confirmés par une méta-analyse plus récente et après ajustement pour les facteurs confondants (le virus de l'hépatite B ou C, l'alcool et le diabète). La magnitude d'effet était plus importante chez les hommes que chez les femmes et chez les patients ayant une infection par le virus de l'hépatite C ou une cirrhose[180].

L'obésité est aussi associée à un risque 4 fois plus élevée de décompensation de cirrhose et d'hospitalisation[181]. Le risque de décompensation de la cirrhose est de 15% chez les sujets avec un poids normal contre 30% chez les patients en surpoids et 43% chez les patients obèses[182].

Certaines études ont montrée un effet additif de l'obésité et de l'alcool sur le risque de développer un CHC. Dans une étude effectuée chez des patients ayant une consommation excessive de l'alcool, l'excès pondéral était un facteur de risque indépendant pour le développement de la cirrhose et de sa décompensation[183]. Au niveau populationnel, les patients qui était obèses et consommaient plus de 15 unités d'alcool par semaine avaient un risque relatif de mortalité liée au foie de 18.9 (95% CI 6.84 – 52.4) vs. 5.30 (95% CI 1.36 – 20.7) chez les obèses qui consommaient < 15 unités d'alcool par semaine. L'excès du risque relatif de mortalité hépatique due à l'interaction de l'alcool et l'obésité était de 5.58[184]. Une analyse du registre américain de transplantation hépatique a démontré que le risque de carcinome hépatocellulaire était trois fois plus élevé chez les patients obeses avec cirrhose alcoolique[185]. D'autres études ont mis en évidence un effet synergique entre la consommation excessive d'alcool et l'obésité ou le diabète[186-188].

Le temps d'exposition aux facteurs de risque métabolique semble avoir un impact significatif sur le risque de développer un CHC à l'âge adulte. Dans une étude récente cas témoin, le risque de développer un CHC à l'âge adulte était 2 fois plus élevé chez les

patients qui avaient des antécédents d'obésité à leur âge de 20 ans [189]. Une autre étude a montré que pour chaque unité d'augmentation de l'IMC par rapport à l'âge, un enfant de 13 ans a une augmentation du risque de CHC de 30% par rapport aux enfants de même âge avec un poids normal[190].

Enfin, l'obésité viscérale semble avoir un effet négatif sur le risque de récidive de CHC après résection chirurgicale (75% vs. 43% à 3 ans)[191]. Toutefois, ces résultats doivent être interprétés avec prudence car n'ont pas été confirmés par des études ultérieures[192].

III – 2. Diabète et carcinome hépatocellulaire

Comme pour l'obésité, le diabète de type 2 est associé avec une augmentation du risque de CHC.

Initialement, il a été considéré que le diabète augmente le risque de CHC uniquement chez les patients ayant d'autres causes de maladie chronique du foie tels que le virus de l'hépatite B, C ou l'alcool[193]. Cependant, dans une large étude de cohorte en Suède, chez 153 852 patients diabétiques, le risque de développer un CHC était 3 fois plus élevé après exclusion d'autres facteurs de risque tels que la cirrhose ou les hépatites virales[194].

Des études ultérieures de cohorte ou cas témoin aux Etats Unis, en Europe ou la région Asie-Pacifique ont montré que le diabète augmente le risque de CHC de 2 à 3 fois indépendamment de la présence et de l'association avec d'autres causes des maladies chroniques du foie[195-198]. Ces résultats ont été confirmés par une méta-analyse incluant 26 études. Parmi 13 études cas-témoin incluses dans cette méta-analyse le diabète était un facteur de risque indépendant de CHC dans 9 études, responsable d'une augmentation du risque relatif de 2.5 fois. Parmi 13 études de cohorte incluses dans

cette méta-analyse, le diabète était un facteur de risque indépendant de CHC dans 7 études (OR = 2.5, 95% CI 1.9 – 3.2).

Le risque de CHC semble être corrélé avec la durée et le contrôle du diabète. Dans une étude américaine, le risque relatif de CHC était significativement plus élevé chez les diabétiques dont la durée de la maladie était de plus de 10 ans (RR = 2.2, 95% CI = 1.2 – 4.8) par rapport à ceux dont l'ancienneté du diabète était de moins de 5 ans[199].

D'autres études ont démontré une relation dose-dépendante entre le taux de glycémie à jeun et le risque de CHC. Une étude réalisée en Autriche a montré que chez les hommes, le risque de CHC augmentait progressivement avec le taux de glycémie à jeun : RR = 2.63, 95% CI 1.15 – 5.99 pour une glycémie entre 5.3 et 6 mmol/l ; RR = 3.5, 95% CI 1.28 – 9.6 pour une glycémie entre 6.1 et 6.9 mmol/l et RR = 4.58, 95% CI 1.81 – 11.6, pour une glycémie à jeun de > 7 mmol/l[200]. De même, il existe une relation dose-dépendante entre l'insulinémie à jeun et le risque de CHC. Une étude collaborative français a montré une corrélation importante entre le risque de CHC, l'insulinémie à jeun (RR = 2.72, 95% CI 1.87 – 3.94) et l'insulinémie 2 heures après le test de charge en glucose (RR = 3.41, 95% CI 2,23 – 5.21). Cette relation a été observée pour le CHC mais pas pour les autres types des cancers[201]. Ces résultats soulignent le rôle de l'hyperinsulinémie dans la pathogenèse du CHC.

Le diabète a un effet synergique avec d'autres facteurs de risque tels que la consommation excessive d'alcool ou le virus de l'hépatite B ou C. Hassan et al., ont démontré que le risque relatif de CHC augmente significativement chez les patients diabétiques qui consomment > 80 g d'alcool par jour (OR = 9.9, 95% CI 2.5 – 39.3) par rapport aux patients qui avaient un seul facteur de risque (OR = 2.4, 95% CI 1.3 – 4.5 chez les diabétiques sans consommation excessive d'alcool et OR = 2.6, 95% CI 1.4 – 4.9 chez les patients avec une consommation excessive d'alcool non diabétiques)[186]. Une

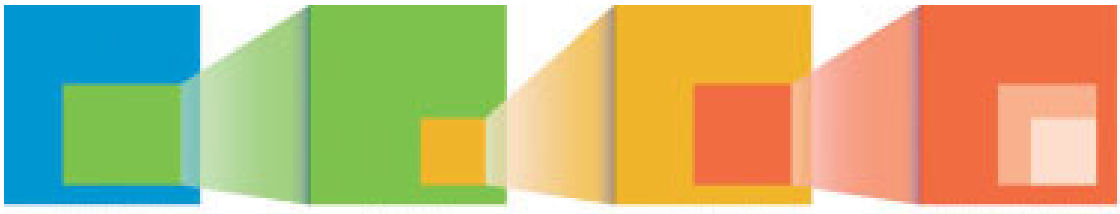
autre étude taiwanaise montre une augmentation de plus de 100 fois du risque de CHC chez les patients avec une hépatite virale B ou C qui étaient diabétiques et obèses[202].

Enfin, le risque de décès dû au CHC est 2 fois plus élevé chez les patients diabétiques[160]. Le risque de récidive de CHC chez les diabétiques est plus important que chez les patients sans diabète. Ce risque devient évident à partir d'un an et augmente progressivement. La proportion des patients sans récidive à 5 ans était de 16.7% chez les diabétiques vs. 36.6% chez les patients sans diabete[203].

III – 3. NAFLD et carcinome hépatocellulaire

Entre 8% et 25% des patients avec NASH évolueront vers la cirrhose et parmi ces derniers 10% à 25% sont à risque de développer un carcinome hépatocellulaire (CHC). Compte tenu de la prévalence actuelle de la NAFLD dans la population générale (30% en moyenne, i.e. 90 millions personnes) il résulte qu'environ 200 000 à 500 000 individus sont potentiellement à risque de développer un carcinome hépatocellulaire dû à la NASH, (**Figure 11**)[204].

Figure 11. Estimation de l'évolution de la NAFLD vers la cirrhose et le CHC aux Etats Unis (adaptée après Siegel et al.).



Des études rétrospectives analysant l'histoire naturelle des patients avec NAFLD ont identifié des cas isolés des CHC chez les patients ayant une cirrhose. La prévalence de CHC dans ces études varie entre 4% et 27%[150, 204]. Dans les études prospectives, la prévalence du CHC varie entre 0% et 0.5% chez les patients avec stéatose et 12.8% chez les patients avec NASH[128, 163, 165]. L'incidence du CHC semble être plus basse chez les patients avec NAFLD que chez les patients avec hépatite C. Dans une étude comparant 247 patients avec NAFLD et 264 patients avec hépatite C, l'incidence du CHC chez les patients avec hépatite C était 6.8% vs. 2.4% chez les patients avec NAFLD[152]. Ces résultats ont été confirmés par Acha et al., qui retrouvent une incidence annuelle du CHC de 4% chez les patients avec une hépatite C vs. 2.6% chez les patients ayant une NAFLD. Les patients avec NASH qui consommaient des quantités modérées d'alcool avait un risque plus élevé de développer un CHC (OR = 3.6, 95% CI 1.5 – 8.3)[205].

Cependant, la proportion réelle des cas de CHC attribuables à la NAFLD est probablement sous-estimée pour au moins 2 raisons : (1) la méconnaissance de la NAFLD et le diagnostic tardif de la maladie, souvent étiquetée comme cirrhose cryptogénétique ; (2) la coexistence de la NAFLD avec d'autres causes des maladies chroniques du foie. Bugianesi et al. ont montré que parmi 641 cas de CHC, 7% avaient une cirrhose cryptogénétique[206]. Globalement il est estimé que 30% à 40% des cas de CHC surviennent chez des patients ayant une cirrhose cryptogenetique[207].

Dans une étude américaine analysant 4406 cas de CHC, la NAFLD seule ou associée à d'autres causes de maladie chronique du foie était le principal facteur de risque de CHC chez 59% des patients, suivi par le diabète type 2 chez 36% des patients et le virus de l'hépatite C chez 22% des patients[208]. Des résultats similaires ont été rapportés par une étude européenne : la NAFLD était responsable de 35% de cas de CHC

et les facteurs de risque métabolique étaient présents dans 66% des cas[209]. Actuellement le CHC lié à la NAFLD est la 2éme cause de transplantation hépatique aux Etats Unis[154].

III-3.1. Particularités du CHC chez les malades avec NAFLD

Dans la plupart des études, les patients avec CHC développé dans le contexte de la NAFLD sont plus âgés et ont plus de comorbidités. Le diagnostic de CHC est souvent tardif car ces patients bénéficient moins des stratégies de dépistages et de surveillance. Aux Etats Unis et en Europe, parmi les patients avec CHC développé sur cirrhose NASH, uniquement 20% à 23% ont bénéficié d'une stratégie de surveillance et de dépistage par échographie et dosage d'alphafetoproteine[208, 209].

Le taux d'alphafetoproteine est souvent < 20 ng/ml chez les patients avec NASH et CHC (47.5% vs. 35% chez les patients avec hépatite C)[210]. En revanche, d'autres biomarqueurs tels que des-gamma carboxy prothrombin sont plus souvent exprimés chez les patients avec CHC et NASH que chez les patients avec l'hépatite C[211].

Une particularité importante du CHC chez les malades avec NASH est la survenue en l'absence de la cirrhose, parfois secondaire à la transformation maligne d'adénomes hépatiques. La proportion des CHC développés en l'absence de la cirrhose varie entre 23% et 55% en Europe, aux Etats Unis et au Japon[208-210, 212]. Une analyse anatomopathologique des tumeurs développées sur un foie non-cirrhotique révèle que ces tumeurs sont de plus grande taille, sont mieux différenciées et moins souvent encapsulées que les tumeurs survenant sur un foie de cirrhose. Dans cette étude, 25% des tumeurs développés en l'absence de cirrhose étaient des adénomes hépatiques avec une transformation maligne[213].

Les patients avec CHC et NASH ont moins accès au traitement curatif en raison d'un âge plus avancé, des multiples comorbidités et du diagnostic tardif dans un stade avancé de la maladie. Dans une série récente américaine, seulement 5.8% des CHC sur

NASH ont été classés stade BCLC A. Parmi les patients avec CHC et NASH, seulement 10.8% des cas ont bénéficié d'un traitement curatif (transplantation ou résection) vs. 22% des patients avec CHC et hépatite C[210].

III – 4. Mécanismes de la carcinogenèse

La cirrhose est considérée comme un état pré-néoplasique et représente en elle même un facteur de risque majeur pour le CHC quelque soit l'étiologie de la maladie chronique du foie. L'agression des hépatocytes par l'agent étiologique (alcool, virus, résistance à l'insuline) va conduire à terme à une altération des séquences nécrose/apoptose/réparation cellulaire et à l'altération de l'expression et de la fonction des protéines contrôlant le cycle cellulaire. Ces mécanismes vont déterminer des lésions de l'ADN et une prolifération cellulaire compensatrice en réponse au stress oxydatif et aux altérations mitochondriales, aboutissant au final à la transformation tumorale[214].

Cependant, dans la NAFLD, le carcinome hépatocellulaire survient souvent en absence de la cirrhose[213]. Cela suggère que d'autres mécanismes propres à la NAFLD tels que l'insulinorésistance, le stress oxydatif, les adipokines et les cytokines pro-inflammatoires ou des voies de signalisation spécifiques (NF-kB, JNK ou PTEN) interviennent dans le développement du CHC chez ces malades en plus des mécanismes classiques de la carcinogenèse.

III-4.1. L'hyperinsulinémie et le carcinome hépatocellulaire

L'hyperinsulinémie intervient dans la carcinogenèse hépatique par des mécanismes directs et indirects.

Des travaux récents montrent qu'il existe une altération quantitative (surexpression chez 40% des cas de CHC) et qualitative du récepteur à l'insuline (IR, insulin receptor) au cours de la carcinogenèse hépatique. Ainsi dans la majorité des tumeurs hépatiques il existe une production préférentielle de l'isoforme A (IR-A) au détriment de l'isoforme B (IR-B) qui est exprimée majoritairement dans le foie sain non-tumoral[215]. IR-A possède aussi la capacité de lier avec une forte affinité l'IGF-2 (insulin growth factor 2) avec des effets mitogéniques et anti-apoptotiques[216].

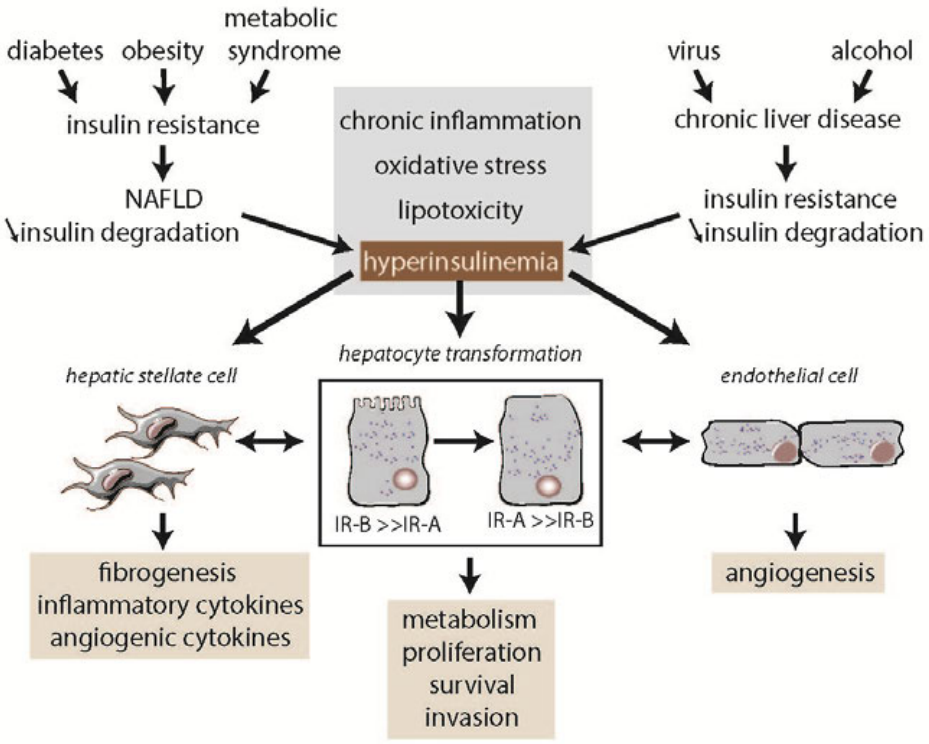
Il a été aussi rapporté une surexpression de l'IRS (insulin receptor substrate) permettant d'amplifier la voie de signalisation insulinique dans les cellules de CHC ainsi qu'une perte d'expression des protéines SOCS (supressor of cytokine signaling) qui sont des régulateurs négatifs de la voie de signalisation insulinique[217].

Le concept de l'inactivation sélective de certaines voies de signalisation de l'insuline émerge de plus en plus comme mécanisme intervenant dans le développement du CHC[218]. Le CHC est une tumeur lipogenique. L'activation de la lipogenèse par l'hyperinsulinémie favorise aussi les effets mitogéniques de l'insuline. Des travaux expérimentaux ont démontré que l'abrogation de la synthèse lipidique (via acetyl coenzyme A carboxylase, stearoyl CoA desaturase ou SREBP – 1c) diminue la prolifération cellulaire[219].

En dehors de ces mécanismes directs, l'hyperinsulinémie intervient dans le développement du CHC par des mécanismes indirects, favorisant la production des cytokines pro-inflammatoires, l'angiogenèse, la fibrogènese et l'activation des cellules étoilées hépatiques (Figure 12)[218].

Au plan clinique, le rôle de l'hyperinsulinémie dans le développement du CHC a été confirmé par une étude française chez 6237 sujets, montrant une relation dose-dépendante entre l'insulinémie et le risque relatif de CHC[201].

Figure 12. Le rôle de l'hyperinsulinémie dans le développement du CHC



III-4.2. Le rôle des cytokines pro-inflammatoires et des adipokines.

L'augmentation des cytokines pro-inflammatoires tels que le TNF-alpha et IL-6 génère une inflammation chronique hépatique mais favorise aussi une prolifération hépatocytaire compensatrice fournissant un environnement permissif pour le développement tumoral[220].

L'adiponectine est peu exprimée dans le CHC mais son expression a une corrélation négative avec la taille tumorale et le risque de récidive locale[221]. Il semble qu'il existe une production tumorale de leptine, corrélée avec la prolifération, la migration et l'invasion des cellules de CHC et avec des tumeurs plus agressives[222].

III-4.3. Les voies de signalisation NF-kB, JNK et PTEN.

Des études expérimentales suggèrent que l'activation hépatocytaire de NF-κB exerce un effet protecteur pour la NASH et le CHC, tandis que l'activation

macrophagique de NF-κB pourrait contribuer à la progression tumorale[223].

JNK1 (c-Jun amino terminal kinase 1) appartenant a la famille de MAPK (mitogen-activated protein kinase) est surexprimé dans l'obésité, la NASH et le CHC. Les AGLs, le TNF alpha et le stress oxydatif contribuent à l'activation de la voie JNK1 dans la NAFLD. Des modelés expérimentaux montrent que l'inactivation de la JNK1 protège contre la NASH et le CHC[224]. Un des mécanismes expliquant le rôle de la JNK1 dans le développement du CHC est l'activation du complexe mTOR (mammalian target of rapamycin), dont le rôle dans la carcinogenèse est bien établi[225, 226]. Comparativement au foie non tumoral, une surexpression de la JNK1 a été identifiée dans le tissu tumoral dans 55% des cas de CHC[227].

Une altération de l'expression ou de l'activité de l'enzyme PTEN (Phosphatase and TENsin homolog) joue un rôle important dans le développement des hépatopathies stéatosiques non alcooliques et leurs progressions vers le CHC. PTEN intervient dans la carcinogenèse via l'inactivation de la voie de signalisation cellulaire PI3K/AKT/mTOR qui a un rôle majeur dans le contrôle de la prolifération cellulaire et de l'apoptose. Contrairement à d'autres suppresseurs de tumeurs dont la perte totale d'expression ou d'activité est requise pour promouvoir la carcinogénèse, l'activité anti tumorale de PTEN est significativement altérée lorsque 30 – 50% de son activité est perdue[228, 229].

III-5. Stratégies de surveillance et prévention du CHC chez les patients avec NAFLD

Les sociétés savantes recommandent une stratégie de surveillance et dépistage du CHC par échographie et dosage de l'aphafetoproteine tous les 6 mois chez les patients ayant une cirrhose[230]. Toutefois, chez les patients avec NAFLD, le CHC survient souvent (25% a 55% des cas) en l'absence de la cirrhose. A présent il n'existe pas une stratégie de surveillance et dépistage du CHC suffisamment validée chez les patients non

cirrhotiques. Les facteurs de risque de CHC les mieux identifiés chez les patients non cirrhotiques sont l'âge avancé, le diabète, l'obésité et le sexe masculin. Probablement que ces malades à haut risque justifieraient d'un dépistage de CHC même dans l'absence de cirrhose, mais une telle stratégie n'a pas encore été validée.

Certains auteurs, ont proposé des modèles prédictifs pour le risque de développer un CHC combinant l'âge, l'IMC, le sexe féminin et le polymorphisme de l'adiponutrine (PNPLA3) dans la formule suivante :

Age x 0.05085–1.88790 x sexe féminin + IMC x 0.09712 + rs738409 (GG) x 0.78377.

Les patients avec un score < 5 étaient considérés à bas risque et ceux avec un score > 7 à haut risque de CHC[231]. Ce modèle a été validé chez les patients avec cirrhose alcoolique mais son applicabilité chez les patients avec NAFLD et particulièrement dans l'absence de la cirrhose n'a pas été validé.

Une stratégie pour diminuer le risque de CHC chez les patients avec NAFLD serait d'agir en prévention sur les facteurs de risque modifiables (i.e. l'obésité et le diabète).

A présent il y a très peu d'études qui ont évalué l'effet de la perte du poids sur le risque de CHC. Une étude récente a démontré que l'adhérence à la diète méditerranéenne pourrait résulter dans une réduction de 50% du risque de CHC[232]. Des études prospectives sont nécessaires afin de mieux analyser l'impact de la diète et de l'exercice physique sur le risque de CHC.

Le metformine n'a pas prouvé son efficacité dans l'amélioration des lésions histologiques chez les patients atteints de la NAFLD[233]. Toutefois, des données expérimentales suggèrent que le metformine pourrait avoir un effet bénéfique et diminuer le risque de CHC via l'inactivation de la voie de signalisation mTOR par plusieurs mécanismes : (1) l'inhibition de la phosphorylation oxydative mitochondriale

et l'inactivation de l'AMPK ; (2) l'amélioration du contrôle glycémique et l'inhibition de l'IGF[234]. Dans une méta-analyse incluant 22 650 cas de CHC observés chez 334 307 patients diabétiques, le risque de CHC était diminué de 50% chez les patients sous metformine. En revanche, les sulfamides hypoglycémiants ou l'insuline étaient associées avec une augmentation de 62% et 161% du risque de CHC[235]. L'effet négatif des sulfamides et de l'insuline est probablement dû à l'hyperinsulinémie.

Des études expérimentales chez des souris transgéniques suggèrent un effet inhibiteur des glitazones sur la carcinogenèse hépatique[236]. L'administration des glitazones semble être associée avec une réduction de 70 % du risque de CHC[199]. Une autre étude montre que la magnitude d'effet est plus importante pour la metformine que pour les glitazones[237].

La prescription des statines diminue le risque de CHC de 25% à 40% avec une tendance pour un moindre risque avec des doses plus élevées et une durée plus longue du traitement mais au final ces différences n'étaient pas statistiquement significatives. Une méta-analyse récente qui montre une réduction du risque de CHC de 37% chez les patients sous statines[238]. L'effet protecteur envers le CHC n'a pas été observé pour les fibrates[239]. Plusieurs mécanismes ont été proposés afin d'expliquer l'effet bénéfique des statines sur le risque de développement du CHC : (1) l'inhibition de la prolifération cellulaire et de la production de collagène par les cellules étoilées hépatiques[240] ; (2) l'induction de l'apoptose[241] ; (3) l'inhibition de l'angiogenèse[242].

En conclusion, le contrôle des facteurs de risque métabolique résulte en une réduction significative du risque de CHC. Des essais prospectifs chez des patients avec NAFLD suivis à long-terme sont nécessaires afin de confirmer ces résultats.

Chapitre IV. La NAFLD et maladie cardiovasculaire

La maladie cardiovasculaire se situe parmi les premières causes de décès aux Etats-Unis et était responsable de 800 937 (30.8%) décès en 2013 par rapport à 600 113 (23%) décès liés au cancer et 48612 (2%) décès liés au foie (http://wonder.cdc.gov/mortSQL.html). Chaque année, environ 635 000 américains développent un évènement coronarien incident et 300 000 un évènement coronarien récurrent. Le coût annuel en 2011 pour les maladies cardio- et cerebrovasculaires était de 320 bilions $, dont coûts directs 196 bilions $ et indirects 124 billions $[243]. La mortalité et morbidité cardiovasculaire sont fortement liées à l'augmentation de la prévalence de l'obésité, du diabète et du syndrome métabolique.

Globalement, la prévalence de l'obésité au niveau mondial a doublé depuis 1980[244]. Actuellement 69% des américains et 50% des européens sont en surpoids ou obèses. Des analyses récentes suggèrent que jusqu'en 2030 la prévalence de l'obésité va augmenter de 33% et que le surpoids et l'obésité vont toucher 3.3 milliards d'adultes[245]. La prévalence du diabète de type 2 était de 6.4% (285 millions personnes) en 2010 ; des estimations récents prédisent une augmentation à 7.7% jusqu'à 2030 (439 millions personnes)[246]. Les évènements cardiovasculaires surviennent 7 à 8 ans plus tôt chez les patients diabétiques[247] et le risque de décès de cause cardiovasculaire est 2 fois plus élevé que chez les patients non diabétiques[160].

La prévalence du syndrome métabolique aux Etats Unis varie de 20% chez les personnes âgés de 20 et 39 ans à 51% chez les personnes de plus de 60 ans[248]. En Europe, la prévalence du syndrome métabolique chez les sujets obeses, varie entre 24% et 65%[249]. Le risque cardiovasculaire est 2 fois plus élevé chez les sujets avec syndrome

métabolique et proportionnel avec le nombre des composantes du syndrome métabolique[247, 250].

IV-1. La NAFLD et la mortalité cardiovasculaire

La NAFLD est fréquemment associée avec la résistance a l'insuline, l'obésité, le diabète type 2, la dyslipidémie et le syndrome métabolique[122, 251] et est considérée actuellement comme un facteur de risque potentiel pour les maladies cardiovasculaires.

La relation entre la NAFLD et la survenue des évènements cardiovasculaires a été analysée dans des nombreuses études rétrospectives, chez des malades avec NAFLD prouvée histologiquement, ou dans des études prospectives, dans la population générale, utilisant des méthodes indirectes de diagnostic (stéatose à l'échographie ou transaminases élevées). Néanmoins, ces études sont très hétérogènes sur plusieurs aspects : (1) le design prospectif ou rétrospectif ; (2) la population incluse: population générale ou sujets avec NAFLD vus dans les centres tertiaires) ; (3) le diagnostic de la NAFLD (biopsie hépatique, stéatose à l'échographie, transaminases ou GGT élevées, marqueurs non invasives) ; (4) critères de jugement principal (survenue des évènements CV non fatales ou mortalité CV); (5) présence des nombreux facteurs confondants, en particulier la consommation excessive d'alcool ; (6) l'ajustement pour les facteurs de risque CV classiques.

Plusieurs études rétrospectives ont démontré que la maladie cardiovasculaire se situe parmi les 3 premières causes de décès chez les malades avec NAFLD avec des taux de mortalité entre 12% et 30% sur une période de suivi qui varie entre de 8 et 21 ans[128, 163-165].

Le **Tableau 6** résume les principales études rétrospectives qui ont évalué la relation entre la NAFLD et la mortalité globale et spécifique[128, 163-165, 168, 252, 253].

Tableau 6. Les principales études rétrospectives qui ont évalué la relation entre la NAFLD et la mortalité globale et spécifique.

Auteurs, Année	Pays	Population d'Etude	Nombre des sujets	Méthode de diagnostique	Suivie (ans)	Résultats
Matteoni, 1999	Italie	NAFLD et transaminases élevées	132	Biopsie hépatique	8.3	Diminution de la survie globale chez les patients NASH vs. stéatose isolée. Survie sans évènements CV similaire entre les 2 groupes.
Dam Larsen, 2004	Danemark	NAFLD (stéatose isolée)	109	Biopsie hépatique	16.7	Survie globale similaire avec la population generale. La maladie CV était la 1-ere cause de décès parmi les patients avec NAFLD*.
Adams, 2005	Etats Unis	NAFLD	420	Biopsie hépatique ou imagerie	7.6	La maladie CV était l'une des causes principales de décès; le taux de mortalité globale était plus élevé chez les patients avec NAFLD vs. la population générale (SMR 1.34; 95% CI, 1.003– 1.76; $P < .03$). Les facteurs prédictifs de décès étaient ; l'âge, le diabète, l'hypertension artérielle, le tabac, la cirrhose et la maladie coronarienne.
Ekstedt, 2006	Suède	NAFLD et transaminases élevées	129	Biopsie hépatique	13.7	La maladie CV était la première cause de décès (15.5% chez les patients avec NASH). Le taux de mortalité globale et CV était 2 fois plus élevé chez les patients avec NASH vs. la population de référence. La mortalité globale n'était pas augmentée en présence d'une stéatose isolée.
Rafiq, 2009	Etats Unis	NAFLD	173	Biopsie hépatique	13	La maladie CV était la première cause de décès (12%). Le taux de mortalité globale était similaire chez les patients avec NASH et stéatose isolée. Le taux de mortalité hépatique était supérieur chez les patients avec NASH. L'âge, le diabète type 2 et la NASH étaient des facteurs prédictifs indépendants de mortalité.
Sodeberg, 2010	Suède	NAFLD	118	Biopsie hépatique	21	La maladie CV était la première cause de décès (30%). Le taux de mortalité globale vs. la population générale était plus élevée chez les patients avec NASH (SMR, 1.9; 95% CI, 1.19-2.76; $P < 0.007$) mais pas chez les patients avec stéatose isolée (SMR, 1.6; 95% CI, 0.98-2.32; P 0.062).
Ekstedt, 2015	Suède	NAFLD	149	Biopsie hépatique	26.4	La maladie CV était la première cause de décès (43%). La fibrose avancée (F3F4) était un facteur de risque indépendant pour la mortalité CV (HR = 4.36, 95% CI 2.29 – 8.29). La NASH san fibrose significative (NAS Score = 5 – 8, F0F2) n'etait pas associee avec une augmentation du risque CV (HR = 1.38, 95% CI 0.72 – 2.65).

Cependant ces études manquent de puissance pour analyser la mortalité spécifique en raison du caractère rétrospectif et du nombre limité de sujets inclus. De plus, ces études ont été réalisées dans des centres tertiaires de référence ce qui limite potentiellement l'applicabilité des résultats dans la population générale. Le syndrome métabolique peut être un facteur confondant important pour le risque CV, or ces études n'ont pas analysé la relation entre la NAFLD et la maladie CV après ajustement pour les facteurs de risque métabolique. Cependant, les études rétrospectives ne peuvent pas répondre si la NAFLD est un facteur de risque CV indépendant, ou l'association avec la maladie CV est due uniquement à des facteurs de risque communs.

En revanche, la plupart des études prospectives montre une augmentation significative de la mortalité CV chez les malades avec NAFLD après ajustement pour les facteurs de risque CV classiques et le syndrome métabolique.

Dans Valoplicella Heart Diabetes Study, Targher et al., montrent que le risque de mortalité CV est deux fois plus élevé chez les patients diabétiques après ajustement pour les facteurs de risque CV classiques. En revanche, deux larges études américaines menées dans la population générale (NHANES III 1988 – 1994) ne retrouvent pas une association entre la NAFLD et la mortalité globale ou spécifique (i.e. CV et hépatique)[254, 255]. Les résultats de ces deux études peuvent être expliqués par des biais méthodologiques: (1) des patients avec « stéatose minime » étaient inclus dans le groupe contrôle alors que l'échographie a une sensibilité limité pour déceler la stéatose de moins de 33% ; (2) certaines des patients dans le groupe contrôle ont pu développer une stéatose pendant la période de suivi (14.5 ans), ce qui peut expliquer l'absence de différence significative entre les deux groupes à l'analyse finale. Ces hypothèses sont renforcées par une étude récente réalisée dans la même population (NHANES III). En plus de l'échographie cette étude a utilisée des marqueurs non invasifs de fibrose (APRI,

NAFLD Fibrosis Score et FIB-4). Comme les deux études précédentes, la stéatose échographique n'était pas associée avec une augmentation des taux de mortalité globale ou CV. En revanche, la fibrose avancée était un facteur prédictif indépendant pour la mortalité globale et CV[256]. Il a été précédemment décrit que la stéatose régresse avec la progression de la fibrose ce qui peut expliquer l'absence de la corrélation entre la stéatose et la mortalité CV dans ces études[115].

Une autre étude récente a retrouvé une association significative entre NAFLD et les marqueurs précoces d'athérosclérose (calcifications coronariennes) mais pas avec les évènements CV cliniques. Ces résultats peuvent être expliqués par l'incidence réduite des évènements CV et l'âge relativement jeune des participantes dans cette étude[257].

D'autres études prospectives ont utilisé l'augmentation des transaminases ou de la GGT comme marqueurs indirects de la NAFLD. Une méta-analyse récente incluant 10 études réalisées dans la population générale, montre que l'augmentation de la GGT était significativement associée avec une augmentation du risque des évènements CV fatals et non fatals après ajustement pour les facteurs de risque CV classiques. L'augmentation d'une unité logarithmique de la GGT était responsable d'une augmentation de 20% du risque des événements coronariens et 54% des événements cerebrovasculaires[258]. Néanmoins, la consommation excessive d 'alcool n'était pas exclue dans certains études et reste un facteur confondant important. Une autre limite de ces études est que la NAFLD peut coexister avec des transaminases normales et donc des patients ayant la maladie ont été possiblement inclus dans le groupe contrôle.

Les **Tableaux 7 et 8** résument les principales études prospectives qui ont évalué la relation entre la NAFLD (stéatose à l'échographie[259],[158, 254, 255, 257, 260-264]transaminases élevées[258, 265-270] ou marqueurs non invasifs[98, 256, 271]) et la mortalité globale et spécifique.

Tableau 7. Etudes prospectives qui ont évalué la relation entre la stéatose à l'échographie ou CT et la mortalité globale et spécifique CV.

Auteurs, Année	Pays	Population d'Etude	Nombre des sujets	Méthode de diagnostique	Suivie (ans)	Résultats
Targher 2005	Italie	Diabète type 2 (Valpolicella Heart Diabetes Study)	744 (NAFLD, 248)	Stéatose à l'échographie hépatique	5	Apres ajustement pour les facteurs de risque CV classiques, NAFLD était un prédicteur indépendant pour la morbidité et la mortalité CV (OR = 1.84, 95% CI 1.4–2.1, P < 0.001) vs. le group contrôle apparié pour l'âge et le sexe.
Targher 2007	Italie	Diabète type 2 (Valpolicella Heart Diabetes Study extended)	2103	Stéatose à l'échographie hépatique	6.5	Apres ajustement pour les facteurs de risque CV classiques, NAFLD était un prédicteur indépendant pour la morbidité (OR = 1.96, 95% CI 1.4–2.7, P< 0.001) et la mortalité CV vs. le group contrôle.
Hamaguchi 2007	Japon	Population générale	1637 (NAFLD, 312)	Stéatose à l'échographie hépatique	5	La NAFLD était un prédicteur indépendant pour les évènements CV après ajustement pour le syndrome métabolique et les facteurs de risque CV classiques (OR = 4.12, 95% CI 1.58 – 10.75, P = 0.004).
Haring 2009	Allemagne	Population générale (Study of Health in Pomerania)	4160	Stéatose à l'échographie et GGT élevée	7.3	Chez les hommes, la NAFLD (définie par la présence concomitante de la stéatose a l'échographie et GGT élevée) avait un taux de mortalité globale (HR = 3.01, 95% CI 1.28 – 3.64) et CV (HR = 6.22, 95% CI 1.22 – 31.62) plus élevé vs. les patients sans NAFLD.
Wong 2011	Hong Kong	Patients a risque CV ayant une indication pour coronarographie	612 (NAFLD, 356)	Stéatose à l'échographie hépatique	1.7	Apres ajustement pour l'âge, le sexe, le diabète, la tension artérielle, les lipides, la NAFLD était un facteur prédictif indépendant pour la maladie coronaire (OR = 2.31, 95% CI 1.46 – 3.64, P < 0.001) mais pas pour la mortalité CV.
Lazo 2011, Stepanova 2012	Etats Unis	Population generale (National Health and Nutrition Examination Survey III)	11613 (NAFLD, 2492)	Stéatose à l'échographie hépatique	14	Les patients avec NAFLD avaient un risque plus élevé de développer de maladie CV non fatale (HR = 1.23, 95% CI 1.04 – 1.44) mais en revanche les taux de mortalité globale, CV et hépatique étaient similaires à la population generale.
Zhou 2012	Chine	Population generale	3324 (NAFLD, 467)	Stéatose à l'échographie hépatique	4	Les patients avec NAFLD avaient une prevalence plus élevée des maladies CV (69% vs. 39%, P < 0.001) ainsi que des taux plus élevés de mortalité CV.
Treeprasertsuk 2012	Etats Unis	NAFLD	309	Stéatose à l'échographie et/ou biopsie hépatique	11.5	Le risque de développer des évènements CV à 10 ans (estimé par le Score de Framingham) était plus élevé chez les patients avec NAFLD. La maladie CV était la 2-eme cause de décès (24%).
Mellinger 2015	Etats Unis	Population générale (Framingham Offspring Study)	3014 (NAFLD, 30%)	CT		La NAFLD était significativement associée avec des signes précoces d'athérosclérose (calcifications coronaires) mais pas avec la survenue des évènements CV cliniques.

Tableau 8. Etudes prospectives qui ont évalué la relation entre la NAFLD (transaminases ou GGT élevées ou marqueurs non invasifs) et la mortalité globale et spécifique CV.

Auteurs, Année	Pays	Population d'Etude	Nombre des sujets	Méthode de diagnostique	Suivie (ans)	Estimation du risque	Commentaires
Wannamethee, 1995	Angleterre	Population générale (British Regional Heart Study)	7613	GGT	11.5	RR = 1.42, 95% CI 1.12 – 1.80	Ajustement pour les facteurs de risque CV, l'alcool et l'IMC)
Ruttmann, 2005	Autriche	Population générale	163 944	GGT	17	Hommes : HR = 1.66, 95% CI 1.40 – 1.98 Femmes : HR = 1.64, 95% CI 1.36 – 1.97	La maladie CV était la 1-ere cause de décès. Ajustement pour les facteurs CV classique, l'alcool et l'IMC.
Lee, 2006	Finlande	Population générale	28 838	GGT	11.9	Hommes : HR = 1.57, 95% CI 1.22 – 2.01 Femmes : HR = 1.44, 95% CI 1.03 – 2.02	Ajustement pour les facteurs de risque CV, l'alcool et l'IMC
Fraser, 2007	Angleterre	Population générale	2961	ALT, GGT	4.6	ALT : HR = 0.96, 95% CI 0.65 – 1.37 GGT : HR = 1.17 (0.93 – 1.48)	Ajustement pour les facteurs de risque CV et l'alcool. Pas de corrélation entre ALT, GGT et le risque CV. Durée de suivie courte (4.6 ans)
Schindhelm, 2007	Pays Bas	Population générale (Hoorn Study)	1439	ALT	10	HR = 1.88, 95% CI 1.21 – 2.92	Ajustement pour les facteurs de risque CV classiques te le syndrome métabolique
Dunn, 2008	Etats Unis	Population générale (NHANES III)	7574	ALT	8.7	HR = 8.15, 95% CI 2.00 – 33.2	Ajustement pour les facteurs de risque CV classiques. Augmentation de la mortalité CV uniquement dans le group d 'âge de 45 – 54 ans.
Yun, 2009	Corée	Population générale	37 085	ALT	5	HR = 2.26, 95% CI 1.22 – 4.19	Ajustement pour les facteurs de risque CV classiques.
Calori, 2011	Italie	Population générale (Cremona Study)	2074	FLI§	15	HR = 1.006, 95% 1.001 – 1.011, p = 0.034	Ajustement pour l'âge, le sexe, le tabac. FLI était prédicteur indépendant pour la mortalité globale, CV et hépatique. La maladie CV était la première cause de décès.

Continuation du Tableau 8. Etudes prospectives qui ont évalué la relation entre la NAFLD (transaminases ou GGT élevées ou marqueurs non invasifs) et la mortalité globale et spécifique CV.

Auteurs, Année	Pays	Population d'Etude	Nombre des sujets	Méthode de diagnostique	Suivie (ans)	Estimation du risque	Commentaires
Kim, 2013	Etats Unis	Population générale (NHANES III data)	11 154	Stéatose a l'échographie ; NFS fibrosis score, FIB4, APRI	14.5	**NFS**: HR, 3.46, 95% CI: 1.91-6.25; **APRI**: HR, 2.53, 95% CI: 1.33-4.83; **FIB-4**: HR, 2.68, 95% CI: 1.44-4.99	La NAFLD n'était pas associée avec une augmentation de la mortalité globale. En revanche, la fibrose était associée avec une augmentation de la mortalité globale et CV.
Perazzo, 2014	France	Diabète type 2 et dyslipidémie	2663	FibroTest, SteatoTest	12.2	Fibrose avancée : HR = 1.92, 95% CI 1.04 – 3.55 ; Progression de la fibrose : HR = 4.8, 95% CI 1.5 – 14.9.	La fibrose avancée estimée par le FibroTest a amélioré la performance du score de Framingham, particulièrement chez les patients à haut risque des évènements CV.

Un autre aspect important concernant l'association entre la NAFLD et la maladie CV est de savoir si le risque CV augmente avec la sévérité des lésions histologiques de la NAFLD (i.e. stéatose isolée, NASH et fibrose). Si tel est le cas, le fait de modifier l'histoire naturelle de la NAFLD (i.e. prévenir la progression ou obtenir la régression) pourrait résulter dans une diminution parallèle du risque CV.

Certains études rétrospectives ont montré une augmentation accrue du risque CV chez les patients avec NASH alors que les patients avec stéatose isolée avaient un risque CV équivalent à la population generale[128, 163, 164]. Ces études n'ont pas analysé l'impact de la fibrose sur le risque CV. Plus récemment, Ekstedt et al., ont analysé l'impact de la NASH (NAS Score 0 – 4 et 5 – 8) et de la fibrose (F0F2 et F3F4) sur la mortalité globale et CV. Dans cette étude, seule la fibrose avancée était associée avec une augmentation du risque CV quelque soit la sévérité des lésions nécroticoinflammatoires alors que la NASH sans fibrose avancée (NAS Score = 5 – 8 et F0F2) n'augmentait pas significativement le risque CV[168].

Dans une autre étude, Perazzo et al., a démontré que la fibrose avancée (estimée par le FibroTest) et la progression de la fibrose étaient corrèles avec une augmentation de l'incidence des évènements CV chez les patients diabétiques de type deux[98]. De plus, la fibrose avancée estimée par le FibroTest a amélioré la performance du score de Framingham, particulièrement chez les patients à haut risque des évènements CV (score de Framingham ≥ 20%).

Ces résultats doivent être interprétés avec prudence. Certes, plus la maladie est sévère, plus le risque d'évènements CV augmente. Toutefois, certains malades avec fibrose avancée ont une NASH « éteinte » dont les lésions typiques (inflammation, ballonisation) ont régressé, ce qui explique la forte association entre la fibrose, mais pas les autres lésions de la NASH, et les évènements CV.

IV – 2. NAFLD et l'épaisseur intima-media

L'épaisseur intima-media est un marqueur précoce d'athérosclérose et des publications récentes suggèrent qu'elle apporte une valeur prédictive du développement des évènements CV supplémentaire à celle des facteurs de risque CV classiques. L'EIMc est associé à une augmentation de 3 à 4 fois du risque d'évènements CV incidents (infarctus de myocarde ou maladies cerebrovasculaires) après ajustement pour l'âge, le sexe et les autres facteurs de risque CV classiques [272-275]. Cependant, l'association entre la progression de l'EIMc et le risque CV est controversée et n'a pas été prouvée dans une méta-analyse récente (PROG-IMT collaborative study)[276]. En revanche, une revue plus ancienne de la littérature incluant 8 études et 30000 sujets suivis pour une période moyenne de 5.5 ans, a montré qu'une augmentation de 0.1 mm de l'EIMc était associée avec une augmentation du risque d'infarctus du myocarde de 15% et du risque de maladies cerebrovasculaires de 18%[277].

L'athérosclérose et la NAFLD partagent des facteurs de risque communs qui font partie du syndrome métabolique : l'obésité, l'hypertension artérielle, le diabète de type 2, la dyslipidémie.

Plusieurs études, la plupart rétrospectives, ont analysée la relation entre la NAFLD et l'épaisseur intima-media (EIMC)[278-298], **Tableau 9**.

Tableau 9. Les principales études qui ont analysé la relation entre la NAFLD et l'EIMC

Auteur, année publication	Type d'étude, Population	Diagnostic de la NAFLD	Prédicteurs indépendant de l'EIMC
Brea, 2005	Etude cas-témoin NAFLD, N = 40, Contrôle, N = 40	Stéatose a l'échographie	NAFLD (OR = 8.4, 95% CI, 2.49 – 29.4, p = 0.001), âge, ferritine
Volzke, 2005	Population générale (Study of Health in Pomerania) NAFLD = 1261, Contrôle = 2961	Stéatose a l'échographie	NAFLD (OR = 1.31, 95% CI, 1.01 – 1.71, p < 0.05)
Targher, 2006	Centre tertiaire NAFLD = 85, Contrôle (appareillés pour l'âge et le sexe), N = 160	Biopsie hépatique	Sévérité des lésions histologiques de la NAFLD (OR = 1.71, 95% CI, 1.4 – 2.2, p < 0.001 ; âge ; HOMA-IR, syndrome métabolique.
Aygun, 2008	Etude cas-témoin NAFLD, N = 40, Contrôle, N = 40	Biopsie hépatique	NAFLD (ß = 0.46, p < 0.01) et l'IMC
Fracanzani, 2008	Etude cas-témoin NAFLD, N = 125, Contrôle, N = 250	Stéatose a l'échographie et biopsie hépatique (N = 54)	NAFLD (OR = 6.9, 95% CI, 3.5 – 15.6, p = 0.0001), âge, pression artérielle.
Gastaldelli, 2009	Population générale N = 1307	Fatty Liver Index (FLI) ≥ 60	FLI (r = 0.12, p < 0.001), âge, sexe, pression artérielle, cholestérol.
Kim, 2009	Population générale NAFLD = 507, Contrôle = 514	Stéatose a l'échographie	NAFLD avec syndrome métabolique (OR = 2.08, 95% CI 1.19 – 3.66)
Petit, 2009	Diabète type 2 NAFLD = 61, Contrôle = 40	MRS	Age (la stéatose n'était pas corrélée avec l'EIMC)
Ramilli, 2009	Patients adressés pour échographie abdominale. N = 154, NAFLD = 90	Stéatose a l'échographie	NAFLD (OR = 1,85, 95% CI 1.33 – 2.57)
Wang, 2009	Population générale N = 170	Stéatose a l'échographie	Taux d'ALT (OR = 1.44, 95% CI, 1.09 – 189).
Salvi, 2010	Population générale NAFLD = 92, Contrôle, N = 128	Stéatose a l'échographie	NAFLD avec syndrome métabolique
Mohammadi, 2011	NAFLD = 84, Contrôle = 65	Stéatose a l'échographie	NAFLD sans syndrome métabolique
Neri, 2011	NAFLD (N = 42 en hémodialyse et 49 avec fonction rénale normale) Contrôle, N = 60	Stéatose a l'échographie, biopsie hépatique	NAFLD quelque soit la fonction rénale. Les patients en hémodialyse avec NAFLD étaient plus a risque

Continuation du Tableau 9. Les principales études qui ont analysé la relation entre la NAFLD et l'EIMC

Auteur, année publication	Type d'étude, Population	Diagnostic de la NAFLD	Prédicteurs indépendant de l'EIMC
Valenti, 2011	NAFLD, N = 506	Stéatose a l'échographie, biopsie hépatique	Age, pression artérielle, glycémie a jeun, LDL, IMC, ferritine
Huang, 2012	Population generale NAFLD = 2590, Contrôle = 60420	Stéatose a l'échographie	NAFLD (OR = 1.35, 95% CI 1.06 – 1.32, p = 1.015)
Kang, 2012	NAFLD, N = 320 Contrôle, N = 313	Stéatose a l'échographie	NAFLD avec (OR = 1.17, 95% CI 1.05 – 1.31, p = 0.003) ou sans syndrome métabolique (OR = 1.23, 95% CI 1.02 – 1.46, p = 0.016)
Thakur, 2012	NAFLD sans diabète, N = 40 Contrôle, N = 40	Stéatose a l'échographie	NAFLD (OR = 4.8, 95% CI 1.8 – 12.8)
Kozakova, 2012	Population générale, N = 1012	Fatty liver Index (FLI) ≥ 60	FLI (ß = 0.15 ± 0.04, p < 0.0005), âge, sexe, pression artérielle systolique, LDL, antécédents familiaux des maladies CV
Kim 2013	Population générale, NAFLD = 320 Contrôle = 449	Stéatose a l'échographie	NAFLD, chez les femmes avec transaminases élevées
Petta, 2013	Centres tertiaires NAFLD = 162 + 267 Pas de groupe contrôle	Biopsie hépatique	PNPLA3 chez les sujets de < 60 ans (OR = 6, 95% CI 1.36 – 29, p = 0.01). PNPLA3 était associée avec la progression de l'EIMC
Kim 2014	Diabète type 2 NAFLD = 3226 Contrôle = 1211	Stéatose a l'échographie	NAFLD avec résistance a l'insuline

Dans une méta-analyse récente ayant inclus sept études évaluant la relation entre la NAFLD et l'EIMC, les patients avec NAFLD avait une augmentation de 13% de l'EIMC par rapport au groupe contrôle sans NAFLD[299]. Les lésions d'athérosclérose semblent survenir 5 à 10 ans plus tôt que dans la population generale[283]. La plupart de ces études ont été réalisées chez des sujets caucasiens. Huang et al., a rapporté dans une étude réalisée dans la population générale en Chine, que les sujets avec stéatose à l'échographie avaient une EIMC significativement plus élevée par rapport aux sujets sans stéatose, après ajustement pour les facteurs de risque conventionnels[293].

Dans certaines études, la NAFLD était un facteur prédictif indépendant de l'EIMC après ajustement pour les facteurs de risque communs[278, 285, 290, 293, 294]. La NAFLD semble être associée à l'EIMC au delà de l'association fréquente avec le syndrome métabolique. Dans l'étude cardio-GOOSE, Salvi et al., a montré que l'EIMC était significativement plus élevée chez les patients avec NAFLD et syndrome métabolique par rapport aux patients avec NAFLD sans syndrome métabolique[289]. Des résultats similaires ont été rapportés par Kim et al., dans une étude transversale sur 1021 sujets dans la population générale en Corée (OR = 2.08, 95% CI 1.19 – 3.66 chez les sujets avec NAFLD et syndrome métabolique vs. OR = 1.18, 95% CI 0.64 – 2.19 chez les sujets avec NAFLD sans syndrome métabolique)[285].

Certains aspects concernant l'association entre la NAFLD et l'athérosclérose carotidienne doivent être soulignées. (1) Toutes ces études ne peuvent pas adresser la relation entre la NAFLD et l'athérosclérose carotidienne en raison de leur caractère rétrospectif et leur design transversal. (2) Il existe une grande hétérogénéité entre les études, notamment pour la définition de la NAFLD. (3) L'association entre la NAFLD et l'EIMC reste sujet de controverse chez les patients diabétiques. Certaines études suggèrent que la NAFLD et plus un épiphénomène, qu'un facteur de risque en soi de

l'athérosclérose[286, 300]. En revanche, Targher et al., a démontré que les patients diabétiques avec NAFLD avaient une augmentation significative du risque de développer de l'athérosclérose carotidienne precoce[281]. (4) L'inflammation semble jouer un rôle important dans les processus pathogéniques communs à la NAFLD et à l'athérosclérose. Certaines études ont montré une association entre la NAFLD et l'EIMC indépendamment des marqueurs systémiques de l'inflammation[278, 300]. Targher et al., a montré que la sévérité des lésions nécroinflammatoires dans la NAFLD était significativement corrélée avec l'EIMC mais aussi avec le syndrome métabolique et la sévérité de l'insulinorésistance[281]. Ces résultats suggèrent un rôle direct de la NAFLD dans la pathogenèse de l'athérosclérose. (5) Il n'existe pas a présent des données analysant l'impact de la transition entre les différents catégories de NAFLD (progression ou régression histologique) et l'évolution des lésions de l'athérosclérose.

IV – 3. Mécanismes pathogéniques de liaison entre la NAFLD et la maladie cardiovasculaire

Les principaux mécanismes communs qui interviennent dans la NAFLD et l 'athérosclérose sont : la dysfonction du tissue adipeux, la résistance à l'insuline et la dyslipidémie « atherogénique », le stress oxydatif et le déséquilibre entre les facteurs pro- et anticoagulants. Le foie est la source mais aussi l'organe cible de ces mécanismes ce qui explique à la fois la progression de lésions hépatiques mais aussi le développement des complications extrahépatiques, particulièrement le diabète type 2 et la maladie cardiovasculaire (**Figure 13**).

La dysfonction du tissu adipeux secondaire aux facteurs environnementaux, diététiques et génétiques, entraîne une résistance à l'insuline périphérique qui va augmenter la lipolyse du tissue adipeux et le flux d'acides gras libres vers le foie. L'accumulation des lipides dans le foie entraîne un cercle vicieux et augmente en plus la résistance à l'insuline. La présence d'acides gras dans le foie est significativement corrélée à l'inflammation et à la fibrose hépatique chez les sujets atteints de la NAFLD[301, 302].

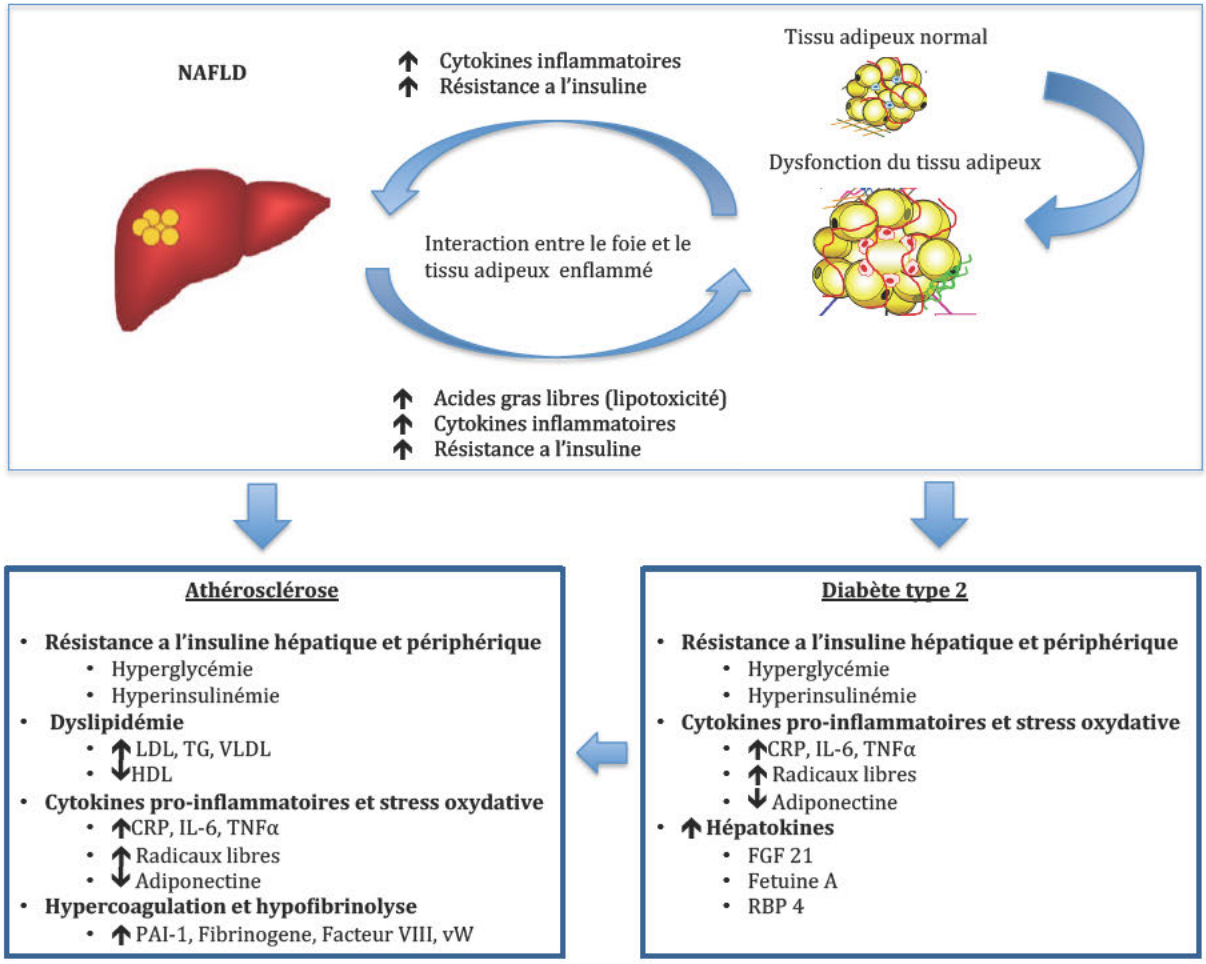
L'inflammation systémique, liée à la présence des molécules pro-inflammatoires circulantes semble avoir un rôle important dans la relation entre la NAFLD et l'athérosclérose. L'activation de la voie NF-kB dans le foie est responsable de la transcription des gènes pro-inflammatoires ce qui entraîne un état inflammatoire chronique. Le tissue adipeux enflammé produit des cytokines pro-inflammatoires (CRP, IL-6, TNF-alpha) qui contribuent à la progression des lésions hépatiques mais aussi à l'athérosclérose[1]. Il a été démontré que les patients avec NAFLD ont des taux plus élevés de protéine C réactive hypersensible, de fibrinogène ou de PAI-1[303]. D'autres auteurs ont confirmé la présence d'un état pro-coagulant (augmentation des taux de fibrinogène, PAI-1, facteurs VIII, IX, XII, VII et von Willebrand) associé a la NAFLD, en corrélation avec la sévérité des lésions histologiques[304].

Les malades atteints de la NAFLD ont un profil particulier de dyslipidémie, dit « atherogénique », qui est caractérisé par des taux bas d'HDL et des taux élevés de triglycérides, VLDL, apoprotéine B100 (apo-B) et LDL (particulièrement LDL oxydée)[305]. Des données épidémiologiques suggèrent qu'une diminution de 1 mg/dl de taux de HDL est associée avec une diminution de 2% a 3% du risque CV[306]. L'apo-B est importante pour l'excrétion des molécules de VLDL. Elle est corrélé avec le syndrome métabolique et a une valeur prédictive positive pour l'athérosclérose et le risque CV[307].

Les différentes études ont rapporté des résultats contradictoires concernant la relation entre la NAFLD et la cinétique de VLDL : diminution de la sécrétion de VLDL-apoB chez les patients obèses avec NAFLD[308] ; augmentation de la sécrétion VLDL-apoB et VLDL-TG chez les patients en surpoids ou obèses avec NAFLD et diabète type 2[309] ; sécrétion normale de TG chez les sujets obèses avec NAFLD[310]. Ces résultats sont probablement dus aux différences de chaque étude entre le groupe contrôle et NAFLD pour les facteurs influençant le métabolisme hépatique des lipoprotéines (tels que le diabète ou le tissu adipeux viscéral).

Un pourcentage de small-dense LDL-cholestérol (sdLDL-C) de plus de 30% est associé avec une augmentation significative du risque CV[311]. Une étude récente a montre que la proportion de sdLDL-C chez les sujets avec ALAT élevées était de 45%. Le même étude montre que les patients avec ALAT élevées et insulinorésistance avaient un profil lipidique très athérogène avec une augmentation significative de sdLDL-C et particules de LDL ainsi qu'une augmentation des triglycérides et de la taille des particules de VLDL[312].

Figure 13. Les mécanismes qui peuvent expliquer la relation entre la NAFLD et la maladie cardiovasculaire (adaptée selon Armstrong et al.)[313]



Chapitre V. Article 1.

A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver.

V-1. Rationnel et objectifs de travail

La stéatose hépatique non alcoolique (NAFLD) regroupe deux entités distinctes : la stéatose isolée (simple fatty liver, NAFL) et la steatohépatite (NASH). La dichotomie entre ces 2 entités s'appuie sur : (1) les critères histologiques, (2) des données expérimentales et (3) l'histoire naturelle de la maladie.

La NAFL est définie par la présence de la stéatose sans ou avec minimes lésions d'inflammation, sans fibrose, alors que la NASH regroupe la stéatose avec des lésions d'inflammation lobulaire/portale et de ballonisation hepatocytaire, avec ou sans fibrose[90].

Des données expérimentales suggèrent que l'accumulation des triglycérides dans le foie est un mécanisme de protection en réponse à l'augmentation d'afflux d'acides gras libres. L'inactivation de l'enzyme finale qui intervient dans la synthèse de triglycérides (diacylglycerol-acyltransferase, DGAT), prévient l'accumulation des graisses dans le foie mais en revanche favorise la formation des métabolites toxiques d'acides gras libres et les mécanismes de lipotoxicité responsables de l'apparition des lésions de nécroinflammation hepatocytaire. L'accumulation des triglycérides et les mécanismes de lipotoxicité peuvent survenir en parallèle mais semblent être indépendantes les unes des autres[1]. Par conséquent, au vu de ces mécanismes, il est attendu que la stéatose isolée ne progresse pas. Les lésions de NASH pourraient être dues à une augmentation d'afflux d'acides gras libres dans le foie dépassant la capacité d'estérification en triglycérides et conduisant à l'accumulation d'espèces lipotoxiques.

Sur le plan clinique, le pronostic de ces deux entités (NAFL et NASH) semble être différent. La stéatose isolée n'augmente pas la mortalité globale ou spécifique. En revanche, la NASH augmente significativement la mortalité globale, soit d'origine hépatique, soit d'origine cardiovasculaires[164].

Cette dichotomie entre la NAFL et la NASH a des conséquences directes sur la prise en charge des patients. Les sociétés savantes recommandent la prise en charge thérapeutique spécifique pour les malades avec NASH, alors que les mesures générales hygiéno-diététiques suffissent pour les malades avec NAFL[7, 8].

A présent, il existe peu des données dans la littérature sur l'évolution histologique à long terme des malades avec stéatose isolée. Des cas isolés de transition d'une simple stéatose vers la NASH ont été décrits, mais l'évolution des malades avec une simple stéatose n'a pas été spécifiquement analysée[126, 128]. Néanmoins, ces résultats mettent en question le dogme classique qui stipule que les malades avec stéatose isolée ont une forme bénigne, sans progression.

L'objectif de notre étude était de mieux comprendre l'histoire naturelle de la NAFLD et d'analyser particulièrement l'évolution histologique des malades avec stéatose isolée. Nous avons également essayé d'identifier les facteurs prédictifs de progression de la maladie d'une stéatose isolée vers une stéatohépatite.

Article 1*:* "A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver",

Research Article

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A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver

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Background & Aims: Disease progression in non alcoholic fatty liver disease (NAFLD) is not well understood and there is controversy about whether non alcoholic fatty liver (NAFL, i.e., steatosis alone or with mild inflammation not qualifying for steatohepatitis) can evolve towards steatohepatitis (NASH) with fibrosis.

Methods: We reviewed 70 patients with untreated NAFLD and with two biopsies performed more than one year apart. Clinical and biological data were recorded at the time of both biopsies. Alcohol consumption did not change during follow up.

Results: Initially 25 patients had NAFL and 45 had NASH and/or advanced fibrosis. After a mean follow up of 3.7 years (s.d. 2.1), 16 NAFL patients developed NASH, eight with severe ballooning and six with bridging fibrosis on the follow up biopsy. Patients with mild lobular inflammation or any degree of fibrosis were at higher risk of progression than those with steatosis alone. Those with unambiguous disease progression were older and had worsening of their metabolic risk factors (higher weight and more diabetes at baseline and during follow up). In the whole cohort, ballooning progression and bridging fibrosis often occurred together and co existed with a reduction in ALT, higher weight gain, and a higher incidence of diabetes during follow up.

Conclusions: A substantial proportion of patients with NAFL can progress towards well defined NASH with bridging fibrosis, espe cially if metabolic risk factors deteriorate. Even mild inflamma tion or fibrosis could substantially increase the risk of progression when compared to steatosis alone. Current monitor ing practices of these patients should be revised.

Keywords: Steatosis; Steatohepatitis; Fibrosis; Insulin resistance; Liver biopsy.
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Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gammaglutamyltranspaptidase; NAS, NAFLD activity score; HOMA-IR, homeostasis model assessment.

Introduction

Patients with NAFLD are increasingly seen in hepatology clin ics, and their management requires decisions on invasive exploration, follow up, and treatment. These decisions are based on our understanding of the natural history of this dis ease, which has so far been largely incomplete. One reason is the inter individual variability in the natural course of the dis ease, a feature shared by many other chronic liver diseases. Another reason is the significant heterogeneity of the clinical presentation. Indeed, NAFLD encompasses both steatohepatitis (NASH), considered the more aggressive form of the disease, and NAFL (non alcoholic fatty liver) grouping isolated steatosis and steatosis with mild lobular inflammation alone [1,2]. NAFL is thought of as a benign, non progressive condition which does not increase overall or liver related mortality [3 5]. In contrast, patients with NASH have a ten fold increase in liver related mortality, die of cirrhosis, and develop liver cancer [3,6 9].

Because of this clear cut dichotomy, NAFL and NASH appear to be very distinct entities, somehow diverging in the early course of the disease. This paradigm was corroborated by exper imental data suggesting that in animal models, triglyceride stor age protects liver cells from fatty acid induced apoptotic pathways [10]; moreover, blocking the final step of triglyceride synthesis by genetic inactivation of diacylglycerol acyltransferase type 2 results in less steatosis, but more inflammation, oxidative stress, and fibrosis [11]. Therefore, the current thinking is that tri glycerides are a form of safe storage of liver fat and therefore patients with steatosis alone are protected from hepatic deterio ration. In this case, there should be little or no transition from steatosis to steatohepatitis, or at least no more than expected from the background noise due to sampling variability of the liver biopsy.

We undertook a study of NAFLD patients with repeat liver biopsies in order to better understand the histological course of

the disease, specifically in relation to the initial findings of NAFL or steatohepatitis. We also aimed at correlating histological changes to changes in the metabolic co morbidities often associ ated with NAFLD. Only patients that were unable to successfully implement lifestyle modifications and thus had persistent NAFLD were included.

Patients and methods

We retrospectively analyzed our histology database between 1998 and 2009 and identified all adult patients diagnosed with primary NAFLD (defined as steatosis ⩾10%) who had undergone a repeat liver biopsy one year or more after the index biopsy. Patients with liver diseases other than NAFLD (drug-induced hepatotoxicity, chronic hepatitis B or C, genetic hemochromatosis, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, α1-antitrypsin deficiency, Wilson's disease, etc.) were excluded. No patient had daily alcohol consumption higher than 30 g (men) and 20 g (women). Patients exposed to drugs that can induce secondary NASH (corticosteroids, amiodarone, tamoxifen) were not included. Patients included in therapeutic trials with follow–up liver biopsy were included if they were administered placebo. No specific dietary interventions or physical activity programs were implemented in these patients.

The main reasons for the follow-up liver biopsy were: persistent ALT elevation along with persistence/aggravation of metabolic risk factors; requirement for inclusion in clinical trials (rosiglitazone, high dose ursodeoxycholic acid, selective anticaspase inhibitors, antiPDE4 inhibitors) and the inability to successfully implement dietary and lifestyle changes. Patients who significantly lost weight during follow-up did not undergo a control liver biopsy.

The following clinical and laboratory data were observed at the time of the index and follow-up biopsy: age, past medical history (hypertension, diabetes, dyslipidemia, coronary artery disease, past medical treatment), daily alcohol consumption, tobacco use, body mass index (BMI), waist circumference, and blood pressure. Laboratory tests included: platelet count, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyltranspeptidase (GGT), fasting glucose and insulin, and lipid profile (cholesterol and tryglicerides). Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR, fasting insulin (mUI/L) × fasting glucose (mmol/L)/22.5).

Histological assessment

Liver biopsy was performed percutaneously under ultrasound guidance. For the purpose of this study, all liver biopsies were reviewed in random order by a single pathologist who was unaware of the patient's identity, clinical and biological data, and sequential order of the biopsies. Biopsies were scored using the scoring system proposed by Kleiner *et al.* [12] including the NAFLD activity score (NAS) and fibrosis staging. Patients were classified as having steatohepatitis (i.e., NASH, defined as steatosis (>5%) co-existing with hepatocellular ballooning and lobular necroinflammation, with or without fibrosis) or as having NAFL. The latter was defined as either steatosis alone (bland steatosis) or steatosis without evidence of ballooning, with spotty lobular inflammation of grade 1 maximum (<2 foci/sox power field) and no fibrosis or fibrosis limited to mild periportal or perisinusoidal fibrosis (stage 1 or 2) [1]. Hence, patients with NAFL had insufficient histological injury to qualify for steatohepatitis [1].

Statistical analysis

Continuous variables were expressed as means (±SD) or medians [IQR], as appropriate and categorical variables as frequency and percentage. Numerical variables were compared using analysis of variance for those that were normally distributed and non-parametric tests, such as the Mann-Whitney *U* test, for those without a normal distribution. Differences between categorical variables were analyzed using the Fisher's exact test. Two-sided *p*-values of less than 0.05 were considered statistically significant. Statistical tests were performed using the NCSS 2007 software (Jerry Hinze, Kaysville, UT).

Results

Study population and overall changes between biopsies

Of the 109 patients fulfilling the inclusion criteria, 32 had been treated with an active drug in a randomized controlled trial and 7 had incomplete clinical or biological data. Therefore, 70 patients were analyzed (Table 1 and Fig. 1). Nine patients had iso lated, bland steatosis (without any ballooning or lobular inflam mation), and 16 patients had steatosis and minimal lobular inflammation, without ballooning or advanced fibrosis. Thus 25 patients had insufficient criteria for NASH and belonged to the NAFL category. Six patients had steatosis without ballooning but with lobular inflammation and advanced fibrosis (bridging fibrosis in 4 and cirrhosis in 2); because advanced fibrosis was present, we conservatively considered that these patients in fact had NASH with missed ballooning lesions due to sampling vari ability of the biopsy. The 39 remaining patients fulfilled typical histological criteria for NASH (Fig. 1). Fifteen of them had bridg ing fibrosis and one had cirrhosis.

The mean time interval between biopsies was 3.4 years (s.d. 2.2, range 1 12 years); in 20 patients (29%), the biopsies were taken more than 5 years apart. Alcohol consumption did not change between the two biopsies except for a female patient that reduced alcohol intake by 5 g/d and a male patient that increased it by 20 g, thus reaching the 30 g/d limit. Mean BMI increased by 0.34 (s.d. 2) kg/m^2 (a mean increase of 1.32 kg). Three patients developed diabetes and three were diagnosed with arterial hypertension during follow up. Thus most patients were stable or experienced only a slight progression in exposure to metabolic risk factors.

Histologically, steatosis progressed by ⩾1 grade in 13 patients (19%) and regressed by ⩾1 grade in 23 patients (33%, $p = 0.08$). Ballooning progressed by ⩾1 grade in 26 patients (37%) and regressed by the same amount in 11 patients (16%, $p < 0.01$). Fibrosis progressed by ⩾1 stage in 20 patients (29%) and regressed by ⩾1 stage in 20 additional patients. Finally, the NAS score increased by ⩾1 point in 29 patients (41%) and decreased by ⩾1 point in 22 patients (31%, $p = 0.29$) (Fig. 2).

Histological course of NAFL

Twenty five patients had NAFL on the index biopsy. Ten of them had no fibrosis at all; 15 had minimal portal or perisinusoidal fibrosis. When compared to the other 45 patients with NASH, patients with NAFL had a lower waist circumference (mean 99 cm *vs.* 104 cm, $p = 0.05$), lower HOMA scores [3.27 (s.d. 1.67) *vs.* 5.77 (s.d. 4.37), $p = 0.03$] and a trend towards less frequent diabetes (26% *vs.* 39%) and arterial hypertension (44% *vs.* 58%). There was no difference in age, sex, BMI, daily alcohol or tobacco consumption, aminotransferase and gamma GT, cholesterol or triglyceride values. The median length of biopsy was 20 mm for both groups. The amount of steatosis (median of 40%) was not different between the two populations.

The histological course of these patients with NAFL is depicted in Fig. 3. After a mean follow up of 3.7 years (s.d. 2.1), hepatocyte ballooning occurred in 16 of the 25 patients, including 8 who pro gressed from none to marked ballooning (grades 0 to 2). There fore, at least these 8 patients (32% of those with NAFL) clearly progressed to NASH during follow up. Importantly, bridging fibrosis developed in six patients (five of whom displayed bal looning on the follow up biopsy). Of these 6 patients, one had no fibrosis initially and 5 had only portal or perisinusoidal fibrosis.

We then thought to determine the histological determinants of disease progression. Table 2 shows individual histological data from the 25 NAFL patients and findings on follow up liver biopsy

Table 1. Study group baseline characteristics (N = 70).

	Mean, %	Median [IQR]
Age (years)	52 ± 10.5	52 (43-58)
Body mass index (kg/m²)	29 ± 3.63	29 (26-32)
Diabetes mellitus, n (%)	24 (35)	
Hypertension, n (%)	36 (52)	
Fasting glucose (mmol/L)	6.5 ± 2.42	5.6 (5-7)
Insuline (μmol/L)	15.82 ± 10.5	14.5 (8.4-19.4)
HOMA-IR (%)	4.86 ± 3.75	3.56 (2.17-6)
Total cholesterol (mmol/L)	5.50 ± 1.2	5 (4.58-5)
Triglycerides (mmol/L)	2.07 ± 1.88	1.6 (1-2.2)
ALT (IU/L)	85 ± 43	79 (51-108)
Alcohol (g/d)	4.4 ± 7.5	0 (0-10)
Tobacco, n (%)	11 (16)	
Length of liver biopsy, mm/ portal spaces (n)	23.4 ± 10.9/ 20 ± 12.3	20/16
Steatosis grade, 0/1/2/3, (%)	0/37/29/34	
Lobular inflammation, 0/1/2, (%)	24/62/14	
Ballooning, 0/1/2 (%)	44/36/20	
Mean NAS score (SD) <3/3-4/>4, (%)	3.6 (1.4) 22/48/24	3 (3-5)
Fibrosis stage, 0/1/2/3/4, (%)	24/26/19/27/4	

IQR, interquartile range.

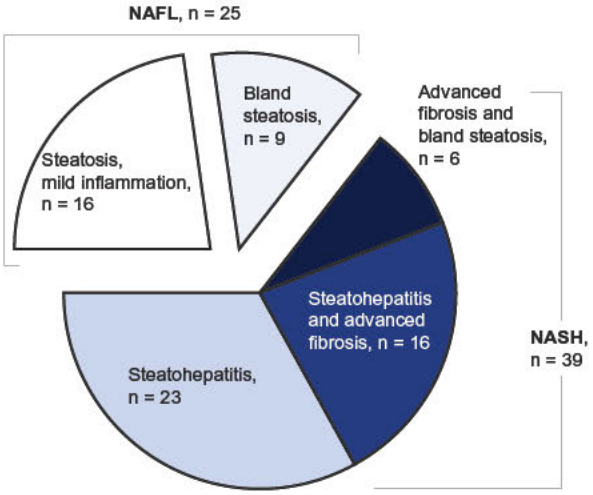

Fig. 1. Baseline histological characteristics. 25 patients had NAFL; 6 patients had steatosis without ballooning, but with bridging fibrosis or cirrhosis; 39 patients fulfilled histological criteria of NASH.

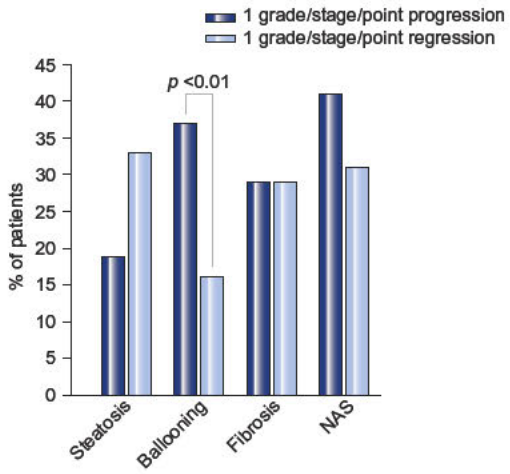

Fig. 2. Overall histological changes. Steatosis progressed in 20% of patients and regressed in 33% ($p = 0.08$); ballooning progressed in 37% of patients and regressed in 16% ($p < 0.01$); fibrosis regressed and progressed by 1 stage in 29% of patients; the NAS score increased or decreased by one point or more in 41% respectively 31% of patients.

with regard to progression to steatohepatitis and bridging fibrosis. Of the 5 patients with steatosis alone (without inflammation, liver cell injury or fibrosis in any location) four did not experience disease progression after a follow up of at least 5 years (Fig. 4). Conversely, of the 16 patients with lobular inflammation, all but three experienced some form of disease progression (Fig. 4). The same was true for seven of the nine patients displaying portal inflammation. Of the 15 patients with minimal fibrosis (either perisinusoidal or portal), 13 experienced some form of disease progression; conversely, 6 out of the 10 without any fibrosis did not progress. Finally, among the 17 patients with inflammation (portal or lobular), only 3 did not experience disease progression (Fig. 4). Collectively, these data suggest that NAFL patients with some form of inflammation or fibrosis are at risk of disease progression whereas this risk is much lower in patients displaying only steatosis.

The mean time between biopsies was not different between these 8 patients with clear progression to NASH and the 17 other patients. The median age was 58 *vs.* 46 years ($p = 0.04$), and there was a trend towards a higher BMI (median 30.1 *vs.* 26.2 kg/m²) and more diabetes (38% *vs.* 18%) at baseline in the group of progressing patients. The metabolic risk factors did not significantly change between biopsies in the latter group. However, there was a trend towards a higher weight gain (median 1.5 kg *vs.* 1 kg) and more frequent diabetes at follow up (50% *vs.* 18%) than in patients without progression. Disease progression occurred despite a trend in ALT reduction (median baseline at 78 IU/L *vs.* 56 IU/L at follow up, $p = 0.2$).

The initial and follow up biopsies from the 25 patients with NAFL underwent another reading by a different pathologist. Only 2 patients out of 25 were not confirmed as having NAFL. Occurrence of ballooning was confirmed in 13 of the 16 patients. Progression to grade 2 ballooning was confirmed in 4 of the 8 patients and progression to bridging fibrosis in 5 of the 6 patients. Thus, qualitatively, the results were largely confirmatory, while numerical differences reflect expected inter observer variability.

Significant disease changes

Occurrence of ballooning

During follow up, ballooning occurred in 21 patients (30%) and resolved in 6 (9%, $p < 0.01$, Fig. 5). In most patients, ballooning occurred without notable weight gain and despite a ⩾25% reduction in ALT (14 patients) and a regression of steatosis (⩾1 grade in all but 4 patients). Interestingly, progression of ballooning often coincided with progression of fibrosis (7/21 patients progressed by ⩾1 stage, including 4 by two stages).

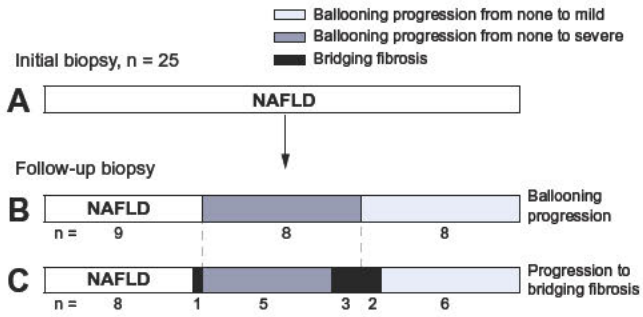

Fig. 3. Histological course of NAFL, individual data. Initial biopsy: (A) 25 patients had NAFLD. Follow-up biopsy: (B) 16 patients had ballooning progression: 8 from none to mild and 8 from none to severe; (C) 6 patients developed bridging fibrosis (black boxes).

The mean interval between biopsies was not different in patients that developed ballooning and those that cleared it. The same holds true for baseline age, sex, BMI, type 2 diabetes, arterial hypertension, daily alcohol or tobacco use and the amount of steatosis at the initial biopsy.

Occurrence of bridging fibrosis

During follow up, bridging fibrosis occurred in 11 patients (16%) and cleared in only 6 cases (9%) (Fig. 5). In patients with bridging fibrosis, six had no or minimal (stage 1) fibrosis at baseline. Again, bridging fibrosis developed together with ballooning progression: at baseline, ballooning was absent/mild in 9 patients and severe in 2; at follow up, it was absent/mild in 4 and severe in 7. There were no significant changes in steatosis or lobular inflammation between baseline and follow up. Progression to bridging fibrosis occurred despite a reduction in aminotransferase values in 7 of the 11 patients.

The mean interval between biopsies was not different between progressors, regressors or patients with no significant changes (3.72, 3.26, and 3.38 years, respectively); age, sex, BMI, and alcohol consumption at baseline were similar. Diabetes was present in 60% of patients with progression to bridging fibrosis *vs.* 30% of those without ($p = 0.06$). Those patients who progressed had a trend toward more weight gain ($\geqslant$2 kg weight gain in 55% *vs.* 36%) and a higher BMI at follow up (median 32.1) *vs.* the others (median 28.5), but the differences were not statistically significant.

Changes in NAS

Eleven patients progressed from a NAS score of 4 or less to a score of 5 or more, including 6 that started at $\leqslant$3. Conversely, only 7 patients regressed from a NAS score of $\geqslant$5 to less than 5, including 3 from $\geqslant$5 to $\leqslant$3 (Fig. 5). In most cases, progression was due to an increase in the ballooning or necroinflammation score and not to changes in the steatosis score. Progression occurred

Table 2. Individual histologic data (index and follow-up) from the 25 patients with initial NAFL.

Patient	Initial biopsy					Follow-up biopsy		Time between biopsies (yr)
	% steatosis	Lobular inflammation	Portal inflammation	Perisinusoidal fibrosis	Portal fibrosis	Steatohepatitis (ballooning grade)	Bridging fibrosis	
1	70	1	1	0	1	Yes (2)	Yes	4.6
2	30	1	1	0	1	Yes (1)	Yes	2.9
3	70	1	0	0	1	Yes (1)	Yes	5.9
4	60	0	0	0	1	Yes (2)	Yes	5.9
5	70	0	0	1	1	Yes (2)	Yes	4.2
6	80	0	0	0	0	No	Yes	5.6
7	70	1	1	0	0	Yes (2)	No	2.1
8	60	1	1	0	0	Yes (1)	No	4.5
9	60	1	1	0	1	Yes (1)	No	2.3
10	40	1	1	1	1	Yes (2)	No	6.7
11	80	1	0	1	0	Yes (1)	No	1.4
12	70	1	0	1	0	Yes (2)	No	4.9
13	70	0	1	0	0	Yes (1)	No	10
14	40	1	0	1	1	Yes (1)	No	3.4
15	40	1	0	0	1	Yes (2)	No	1.6
16	30	1	0	1	1	Yes (2)	No	1
17	20	1	0	0	1	Yes (1)	No	5.1
18	30	1	1	0	0	No	No	1.7
19	20	1	1	0	0	No	No	3.9
20	70	1	0	1	1	No	No	1
21	40	0	0	1	0	No	No	1.1
22	40	0	0	0	0	No	No	5.1
23	30	0	0	0	0	No	No	6.7
24	30	0	0	0	0	No	No	5.9
25	10	0	0	0	0	No	No	5.2

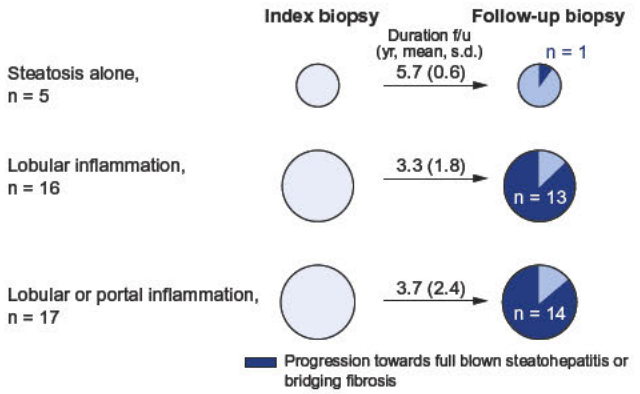

Fig. 4. Disease progression in patients with NAFLD according to the presence of hepatic inflammation on index biopsy. The size of circles is proportional to the number of patients; the dark blue quarters denote patients experiencing histological progression towards full blown steatohepatitis or bridging fibrosis.

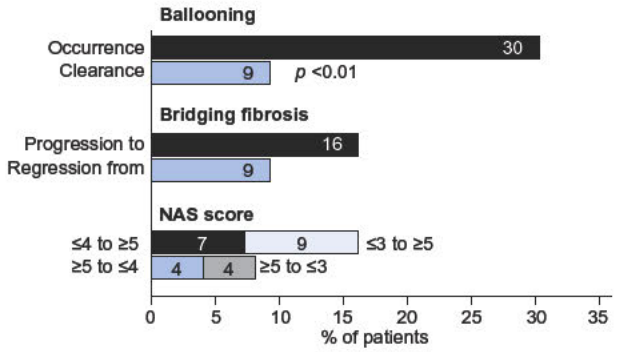

Fig. 5. Significant changes for ballooning (occurrence from none to present; clearance from present to absent) and bridging fibrosis (progression from mild/none to bridging; regression from bridging to mild/none). Changes in NAS. Increase from ⩽4 to ⩾5 (n = 16, including 9 having progressed from ⩽3); reduction from ⩾5 to ⩽4 (n = 8, including 4 with a reduction to ⩽3). Numbers in boxes represent percentages.

despite a trend towards ALT reduction in 8/11 patients (73%), from 86 IU/L (s.d. 41) to 67 IU/L (s.d. 23), $p = 0.09$. There were no significant differences in length of follow up, baseline age, sex, BMI, hypertension, diabetes, alcohol or tobacco use, ALT, GGT, glucose or lipid profile in those patients who progressed *vs.* the others.

Seven patients experienced a reduction in NAS which was mostly due to a decrease in ballooning and necroinflammation. These patients were not different from the 63 other patients for baseline or follow up characteristics. However, they had a lower baseline BMI (mean, 27.7 *vs.* 29.1, $p = 0.33$) and a median weight loss of 2 kg during follow up *vs.* a median weight gain of 1 kg in 63 other patients. One patient progressed to cirrhosis despite NAS reduction over a 5.8 year interval. He developed diabetes and severe arterial hypertension during follow up. On initial biopsy, this patient had mild ballooning and inflammation, grade 3 steatosis, and bridging fibrosis. Interestingly, there was no correlation between changes in NAS and changes in fibrosis ($r = 0.09$, $p = 0.46$).

Factors associated with disease progression

Twenty five patients (36%) had disease progression defined by a composite index which required either progression from NAFL to NASH, occurrence of bridging fibrosis or at least ⩾1 point increase in the NAS score from ⩽4 to ⩾5. These patients were not different from the others as far as clinical, biological, and metabolic characteristics, but had a significantly higher amount of steatosis (median 60% *vs.* 40%, $p = 0.008$). Metabolic risk factors did not significantly change during follow up, but there was a trend towards a higher baseline HOMA IR (5.8 ± 5.1 s.d. *vs.* 3.9 ± 2.5 s.d., $p = 0.09$) in progressors. Disease progression occurred despite ⩾25% ALT reduction. In univariate analysis, only the amount of steatosis on initial biopsy correlated with disease progression ($r = 0.325$, $p = 0.005$). In multivariate analysis, the amount of steatosis was the only independent factor associated with disease progression, even after adjustment for age, sex, BMI, and aminotransferase levels ($r = 0.318$, $p = 0.01$).

Discussion

These findings challenge the current dogma that NAFL is a non progressive condition that rarely, if ever, results in NASH or advanced fibrosis. The dichotomy between NASH, a potentially progressive condition, and NAFL (i.e., steatosis alone or with mild inflammation) that is not associated with adverse hepatic out comes, has until now been the basis of our understanding of the natural history of NAFLD. Early studies on a small number of patients with repeat liver biopsies failed to show any signifi cant changes in patients with steatosis alone [13]. Later reports showed that patients with bland steatosis or steatosis and non specific inflammation have the same survival as the age and sex matched general population [14], while patients with steato hepatitis have increased liver related mortality [15]. The benigni ty of the long term outcome of non alcoholic steatosis [4] was further reinforced by the findings that only one patient out of a series of 170 NAFLD patients with pure fatty liver died of cirrhosis [5]. In striking contrast, steatohepatitis, instead of steatosis, sig nificantly increased liver related mortality ten fold [3,6]. More over, when analyzing ten pathological series with follow up liver biopsy, Argo *et al.* concluded that among histological lesions at the index biopsy, necroinflammation is the only predictor of fibrosis progression, further reinforcing the view that disease progression is dependent on the presence of necroinflammatory lesions which are part of the definition of steatohepatitis.

In this report, which is one of the largest monocentric series of carefully defined patients with NAFL, we show that disease pro gression towards unambiguous steatohepatitis (severe balloon ing) and bridging fibrosis is possible after a rather short follow up of less than 5 years. Although sampling error is a possible con founder (i.e., lobular inflammation and ballooning missed on the initial biopsy), we believe it does not explain the bulk of our find ings; first, because patients with NAFL had a different profile than those with histologically defined steatohepatitis. They were less insulin resistant as measured by surrogate markers and related clinical phenotypes, thus confirming observations made by oth ers a decade ago [16]. Second, because together with the occur rence of steatohepatitis, fibrosis also progressed in some of them from none or very low levels to bridging fibrosis. Since we describe patients evolving from no ballooning to severe bal

looning and from no or mild fibrosis to bridging fibrosis, we believe that these data robustly show genuine disease progres sion and not simply slight changes in histological grades/stages. On a smaller scale, Wong *et al.* [17] reported on three patients out of a series of 13 with mild NAFLD that progressed towards NASH, meaning lobular inflammation and ballooning of any degree, i.e., a lower stringency definition than the one used here, and therefore more prone to sampling variability or inter observer variability. They also report on the case of one individual progressing from a NAS score of <3 to 5 during follow up [17]. However, the NAS is not designed to ascertain the presence of NASH, and the composite score could partly reflect changes in steatosis. Therefore, we believe the present report is the best available demonstration so far of unambiguous disease progression in patients with NAFL.

The number of patients presented here is still limited, and these findings require confirmation in larger series. However, a striking finding is that patients with pure (bland) steatosis with out any inflammation or fibrosis seem to be at lowest risk of dis ease progression. On the other hand, even mild lobular or portal inflammation or fibrosis in any location substantially increases the risk of subsequent diagnosis of steatohepatitis or advanced fibrosis. Thus, in patients with NAFLD, mild lobular inflammation or early perisinusoidal or periportal fibrosis is not trivial but rather indicates a risk of disease progression, particularly if met abolic co morbidities, including overweight, deteriorate. Hence, careful rather than loose monitoring of these patients is advisable and these patients cannot receive definitive reassurance that their hepatic prognosis is benign. These findings are in agreement with the key observation made by Argo *et al.* that necroinflamma tion increases the risk of fibrosis progression in patients with NASH and hence is an important driver of disease progression. Collectively, these data advance our understanding of the natural history of NAFLD by identifying different risk profiles within NAFL patients, the less severe end of spectrum of NAFLD.

While there is still uncertainty about the factors promoting the transition from NAFL to NASH, converging evidence from this series suggests that patients with clear disease progression were older and had worsening of metabolic risk factors (higher BMI, more weight gain, more diabetes) during follow up com pared to those without progression. Large cohort studies have confirmed that the occurrence of steatosis, defined by imaging, was associated with enhancement of metabolic co morbidities associated with insulin resistance [18]; conversely, the resolu tion of steatosis coincided with their regression, quantitatively or qualitatively. Based on current and previous findings, we hypothesize that in some patients with NAFL, the persistence or worsening of insulin resistance and related metabolic risk factors (overweight, diabetes, hypertension, dyslipidemia, etc.) will trigger the occurrence of steatohepatitis. Future studies should aim at better identifying, among the large number of patients with non alcoholic steatosis, those at risk of disease progression. The present findings stress the need to revise cur rent guidelines that do not recommend specific hepatic moni toring in NAFLD patients without NASH [2,19]. Careful hepatic monitoring including insulin resistance surrogates, non invasive fibrosis markers, and potentially a repeat liver biopsy might be necessary in selected patients.

In this series of 70 NAFLD patients with both NAFL and NASH, the overall trend between biopsies was a reduction in steatosis, an increase in the amount of hepatocyte cell injury (ballooning) and, in some patients, progression towards advanced fibrosis. Despite a rather short time interval between biopsies, a propor tion of NAFLD patients clearly experience disease progression. Alcohol consumption was nil or very low in this series and did not increase during follow up. In this selected NAFLD population, drinking alcohol did not explain the histological deterioration.

Interestingly, bridging fibrosis developed together with an increase in hepatocyte ballooning, a feature of hepatic cell injury. This further indicates that steatohepatitis may be a major driving force behind fibrosis progression as suggested by other reports based on serial liver biopsies [20]. Therefore, a reasonable infer ence is that clearance of steatohepatitis might represent a valid therapeutic end point, as currently recommended by expert pan els [19,21].

Of note, in some patients disease progression occurred despite a reduction in ALT levels; while only two ALT measurements were available here, previous reports showed fibrosis progression despite a similar reduction in ALT [17,22]. A spontaneous decline in ALT levels during follow up, especially if the metabolic risk factors are still present (i.e., not concomitant with significant weight reduction), should not falsely reassure patients and health care providers. Finally, as shown in the course of patients with NAFL, there was a trend towards a higher proportion of diabetes and an increase in BMI at follow up in patients with histological deterioration, further strengthening the assertion that underlying metabolic abnormalities increase the risk of progressive disease. Conversely, patients with NAS reduction improved their meta bolic profile.

There are limitations to the design of the present study. This is a retrospective series and not a prospectively designed cohort with systematic control biopsies at fixed time intervals. Therefore, the rate of progression and impact of correlative co morbidities in the course of the disease should be interpreted with caution. All patients were explored in a tertiary referral center with academic interest in this disease, which, arguably, could induce a bias towards more severe cases. Nonetheless, the decision to perform a control liver biopsy was not determined by specifics of the population or findings on the initial biopsy. In fact, in many cases, the biopsy was repeated regardless of the initial severity in order to have a recent histological evaluation for inclusion in therapeutic trials. The population reported here covers the whole spectrum of NAFLD. Moreover, in our institution only patients who significantly lost weight and normalized ALT with diet and lifestyle interventions are not considered for histological evaluation. We therefore believe that, despite the absence of a predefined protocol for repeating the liver biopsy, this population is representative, to a large extent, of all NAFLD patients seen in tertiary centers. Moreover, the persistence of overweight and other metabolic risk factors and the absence of significant changes in alcohol consumption during follow up are also typical of the population at large of subjects with NAFLD, so histological changes captured in this cohort are most probably close to those dictated by the natural course of the disease.

In conclusion, this large series of patients with histological fol low up shows that a fraction of patients with NAFL can unambig uously evolve towards well defined steatohepatitis, and in some of them, bridging fibrosis. Patients with NAFL might not be a homogenous population. The presence of mild lobular inflamma tion or any amount of fibrosis substantially increases the risk of histological progression in the mid term while those with steato sis alone are at lowest risk. Consequently, and pending confirma

tion by future reports, current practices of no or very relaxed hepatic monitoring in patients with steatosis alone might not be appropriate, and a more active, hepatological follow up could be necessary. Patients with disease progression experienced a degradation of clinical features associated with insulin resistance. The potential for histological deterioration in NAFLD patients who do not drink alcohol is not negligible, even after a short follow up.

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Conflict of interest

VR is a consultant for Abbot, Conatus, Galmed, Genfit, Gilead, Ent erome, and Phenex all unrelated to the present manuscript.

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V-2 Résumé de l'article 1.

V-2.1. Méthodes.

Nous avons étudié 70 patients avec NAFLD prouvée histologiquement ayant eu deux biopsies hépatiques réalisées à plus d'un an d'intervalle. L'indication de la biopsie de suivi était: persistance des transaminases élevées concomitante avec l'aggravation des facteurs de risque métabolique ; échec des mesures hygiéno-diététiques ; documentation histologique nécessaire avant inclusion dans des essais thérapeutiques. Toutes les biopsies ont été relues par un seul anatomopathologiste en aveugle pour les données cliniques. La stéatose, la nécroinflammation et la fibrose ont été notés selon la classification de Kleiner et Brunt[85].

La NAFL a été définie par la présence d'une stéatose de > 5% sans inflammation/ou avec inflammation minime (grade 1) en absence de la fibrose.

Nous avons analysé l'évolution globale des lésions histologiques dans l'intervalle de suivi (progression, régression). Nous avons particulièrement analysé l'évolution des patients avec NAFL au début de la période de suivi.

V-2.2. Résultats principaux et discussion

Parmi les 109 patients avec biopsies hépatiques répétées identifiées initialement, 32 ont été exclus en raison de traitements spécifiques pour la NASH dans des essais cliniques dans l'intervalle entre les deux biopsies; 7 autres patients ont été exclus en raison des données cliniques manquantes au moment de la biopsie. 70 patients ont été finalement étudiés. Parmi les 70 patients inclus, 25 ont été diagnostiqués avec NAFL (9

avec stéatose isolée et 16 avec stéatose et inflammation minime sans fibrose) et 45 avec NASH (6 avait une stéatose associée à une fibrose avancée, 39 avait des critères histologiques de NASH, dont 16 avec fibrose avancée). L'intervalle moyen entre les 2 biopsies était de 3.4 ± 2.2 ans (rang de 1 a 12 ans) ; chez 29% des patients les deux biopsies étaient espacées de plus de 5 ans.

Malgré cette courte période de suivi une proportion significative (36%) de patients avec NAFLD ont eu une progression des lésions histologiques : soit progression de NAFL vers la NASH, soit développement de la fibrose en pont, soit l'augmentation du score NAS de ≥ 1 point de ≤ 4 à ≥ 5.

Pendant la période de suivi, 16% des patients ont développé une fibrose avancée (≥ F3). Parmi ces patients, 60% étaient diabétiques et 55% ont eu une prise de poids de ≥ 2 kg. Comme retrouvé dans d'autres études, la progression de la fibrose était associée à une augmentation de la ballonisation et de l'inflammation hepatocytaire[134]. D'autres études suggèrent que la fibrose est la seule lésion histologique corrélée avec le pronostic à long terme des malades atteints de la NAFLD[168]. Cependant, nos résultats montrent que les lésions de nécroinflammation de la NASH précedent la progression de la fibrose. La résolution de la NASH est donc un prérequis pour la résolution à long terme de la fibrose. Par conséquent, les interventions pharmacologiques dans la NAFLD ne doivent pas viser uniquement la régression de la fibrose mais aussi la résolution de la NASH.

Comme cela a été déjà rapporté[125, 130], la progression vers la fibrose en pont dans cette série est survenue malgré une réduction des taux d'ALAT. Par conséquent, la normalisation des transaminases ne doit pas rassurer les médecines et les patients, surtout en cas de persistance ou aggravation des facteurs de risque métabolique.

Seulement 3 patients (4%) ont eu une régression du score de NAS de ≥ 5 a ≤ 3. Des résultats similaires ont été obtenus dans une étude récente où la régression de la

NASH a été rapportée uniquement chez 7% des patients [132]. Ces résultats rappellent ceux obtenus dans les essais thérapeutiques ou la résolution de la NASH faisait partie des critères d'efficacité. Par exemple, dans un récent essai thérapeutique contrôlé randomisé de l'acide obéticholique vs. placebo, la résolution de la NASH était rapportée chez 13% des patients dans le bras placebo vs. 22% dans le bras de traitement (p = 0.08)[70]. Ces résultats suggèrent que dans l'absence d'une intervention thérapeutique spécifique, la NASH est une maladie potentiellement progressive dont la régression spontanée est rare.

Nous avons spécifiquement analysé l'évolution des 25 patients initialement diagnostiqués avec NAFL. Pendant le suivi 32% de ces patients ont développé une NASH (progression de la ballonisation de grade 0 à grade 2) et 24% ont développé une fibrose en pont (progression de stade 0 ou fibrose perisinusoïdale au stade 3). Ces résultats reflètent une réelle progression de la maladie au delà de ce qui peut être expliqué par la variabilité d'échantillonnage de la biopsie hépatique[89]. Il est intéressant de noter que la majorité des patients ayant progressé avaient en plus de la stéatose des lésions d'inflammation lobulaire ou portale ou une fibrose perisinusoïdale. En revanche, la progression vers la stéatohépatite semblait très rare (un seul patient sur 5) en cas de stéatose isolée. Ces résultats confirment ceux rapportés précédemment par Argo et ses colab.. Dans une revue systématique des 10 études longitudinales des patients avec NAFLD suivis par biopsies répétées, les auteurs ont identifié l'inflammation lobulaire comme un facteur de risque indépendant pour la progression des lésions histologiques (pour la fibrose spécifiquement) pendant le suivi à long terme[134].

Jusqu'à présent peu d'études ont analysé l'évolution des patients avec NAFL. Précédemment, Wong et al., ont rapporté trois patients avec stéatose isolée qui ont développé une NASH durant la période de suivi. La définition de la NASH était moins

stricte dans cette étude (i.e. présence de l'inflammation et la ballonisation quel que soit le grade)[130]. Nos résultats ont été confirmés par une étude ultérieure. Dans cette étude, parmi 27 patients avec NAFL, 44% ont développé une NASH et 22% une fibrose en pont dans une intervalle moyen de 6.6 ans[132].

Dans une metaanalyse récente, parmi 133 patients avec NAFL provenant des différentes études, 39% ont eu une progression de la fibrose d'un stade ou plus (9% ont progresse de stade 0 ou 1 au stade 3) et 53% étaient stables durant la période de suivie. La régression de la fibrose est survenue uniquement chez 8% des patients. Alors que la progression moyenne de la fibrose était deux fois plus lente chez les patients avec NAFL (progression d'un stade dans 14 ans) par rapport aux patients avec NASH (progression d'un stade dans 7 ans), la proportion des patients avec une progression rapide de la fibrose était similaire chez les patients avec NAFL (17.2%) et NASH (18.2)[133]. Ces résultats laissent penser que d'autres facteurs que l'histologie sur la biopsie initiale accélèrent la progression fibrosante de la maladie.

Un aspect essentiel de l'histoire naturelle de cette affection est l'identification des facteurs associés à la progression de la fibrose. Si on prend comme exemple un individu avec NAFL à l'âge de 30 ans, pour une vitesse de progression de 1 stade dans 7 ans, il pourra devenir F3 à l'âge de 51 ans et développer une cirrhose vers l'âge de 60 ans. Même si on assume que la plupart des patients vont avoir une progression lente de la fibrose, les chiffres sont alarmants. Il est estimé que 1 sur 10 enfants a une stéatose, souvent associée à des facteurs de risque métabolique[314]. Donc en cas de persistance des facteurs de risque, même à une vitesse de progression d'un stade tous les 14 ans, à l'âge de 60 ans, ces enfants seront à risque d'avoir une maladie hépatique avancée en l'absence d'intervention thérapeutique efficace.

Malheureusement les facteurs de risque cliniques ne permettent qu'imparfaitement l'identification des individus à risque de progression. Dans notre étude, les 32% patients qui on progressé vers la NASH étaient plus âgés (58 vs. 46 ans), avaient un IMC plus élevé (30.1 vs. 26.2 kg/m^2) et avaient plus de diabète (38% vs. 18%). Durant le suivi, les sujets ayant progressé ont présenté une prise pondérale plus importante et ont développé un diabète plus souvent que les patients sans progression.

Dans l'étude de McPherson et al., seul l'algorithme FIB-4 (un marqueur sérique de fibrose calculé à partir de l'âge, ASAT, ALAT et plaquettes) a eu une valeur prédictive, d'ailleurs modeste (AUROC = 0.63, 95% CI 0.51 – 0.76, p = 0.036) pour la progression de la fibrose[132]. Dans une analyse récente de 270 patients avec NAFLD provenant du registre américain (NASH CRN), 16% ont développé une fibrose en pont ou cirrhose. Les facteurs cliniques associés à la progression de la fibrose étaient le diabète, l'index HOMA IR et le syndrome métabolique. La ballonisation hepatocytaire, les corps de Mallory, l'inflammation portale et le score NAS étaient associés aussi avec la progression de la fibrose[138].

L'ensemble de ces résultats suggère que les patients avec NAFL présentant, en plus de la stéatose, des lésions d'inflammation même minimes, sont à risque de progression histologique. Ce risque semble majoré en cas de persistance ou d'aggravation des facteurs de risque métabolique. Contrairement au dogme classique, ces patients peuvent donc évoluer et doivent bénéficier d'un suivi régulier.

Chapitre VI. *Article 2*.

Nonalcoholic Fatty Liver Disease Increases the Risk of Hepatocellular Carcinoma in Patients With Alcohol-Associated Cirrhosis Awaiting Liver Transplants

VI-1. Rationnel et objectifs du travail

La maladie alcoolique du foie (MAF) et la NAFLD sont actuellement les causes les plus fréquentes de maladie chronique du foie. D'une part, l'incidence annuelle de la cirrhose alcoolique en Europe reste élevée mais constante dans les dernières années, entre 11.8 et 26 pour 100 000 personnes/année, entraînant une mortalité à 5 ans de 69%[315, 316]. D'autre part, la prévalence du syndrome métabolique est en constante augmentation, entre 40% et 60% en Europe et Etats Unis, avec des variations en fonction de l'âge et les différents groups ethniques[317]. En raison de leur prévalence élevée dans la population générale, la MAF et la NAFLD peuvent théoriquement coexister. Parmi 7463 individus issus de la population générale, la NAFLD et la MAF coexistaient comme facteurs associés à une fibrose significative dans 66% des cas[318].

Malgré l'effet hépatotoxique connu de l'alcool, uniquement 8% à 20% des patients ayant une consommation excessive de l'alcool développent une cirrhose suggérant que d'autres facteurs intervient dans la progression de la fibrose.

L'alcool et l'obésité semblent avoir un effet additif sur la sévérité de l'atteinte hépatique. La probabilité d'avoir des transaminases élevées est 5 fois plus importante quand les deux conditions sont associées[319]. L'obésité est associée à des lésions plus fréquentes de stéatose et d'hépatite alcoolique aigue, ainsi qu'avec un risque plus important de développer une cirrhose[183].

La stéatose elle même pourrait être une facteur de progression de la fibrose[320, 321]. Il a été précédemment démontré qu'il existe une corrélation entre la stéatose et l'activation des cellules étoilées du foie qui sont responsables de la progression de la fibrose[322].

Au niveau moléculaire, l'augmentation du flux d'acides gras libres vers le foie associée à l'obésité et à l'insulinorésistance entraîne une induction du cytochrome P4502E1 responsable d'une production excessive de radicaux libres et une activation de la peroxydation lipidique[323, 324]. D'autres études ont mis en évidence une augmentation de la production des cytokines pro-inflammatoires (TNF alpha et IL10), secondaire à une inflammation du tissu adipeux liée à l'alcool[325].

L'alcool et le syndrome métabolique sont des facteurs de risque connus pour le développement du carcinome hépatocellulaire. Il a été précédemment démontré que la stéatose et l'obésité augmentent significativement le risque de carcinome hépatocellulaire. Approximativement 30% des cas de carcinome hépatocellulaire sont attribuables à un excès pondéral. Le risque semble être plus élevé en cas d'obésité que de surpoids[179]. Le diabète de type 2 augmente aussi l'incidence et la mortalité liée au carcinome hépatocellulaire[160].

Ces résultats suggèrent que l'association entre le diabète, l'obésité et l'alcool (dont l'effet carcinogène est connu) pourrait avoir un impact significatif sur la carcinogenèse hépatique.

Compte tenu de : (1) la fréquence élevée de la MAF et de la NAFLD, (2) leurs effets additifs potentiels sur la sévérité de l'atteinte hépatique et (3) le fait que les deux conditions peuvent être réversibles en cas d'interventions spécifiques, il est important de mieux comprendre l'intéraction entre la consommation excessive d'alcool et les

facteurs de risque métabolique, ainsi que leur impact sur la sévérité de l'atteinte hépatique.

Dans ce contexte, l'objectif principal de notre étude était d'analyser l'impact de la stéatose et des facteurs de risque métabolique sur le risque de développer un carcinome hépatocellulaire chez les patients transplantés du foie pour une cirrhose alcoolique.

***Article 2*. « Nonalcoholic Fatty Liver Disease Increases the Risk of Hepatocellular Carcinoma in Patients With Alcohol-Associated Cirrhosis Awaiting Liver Transplants »**

Nonalcoholic Fatty Liver Disease Increases the Risk of Hepatocellular Carcinoma in Patients With Alcohol-Associated Cirrhosis Awaiting Liver Transplants

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BACKGROUND & AIMS: **Many patients with alcohol-associated cirrhosis also have diabetes, obesity, or insulin resistance–mediated steatosis, but little is known about how these disorders affect the severity of liver disease. We analyzed the prevalence and prognostic implications of metabolic risk factors (MRFs) such as overweight, diabetes, dyslipidemia, and hypertension in patients with alcohol-associated cirrhosis awaiting liver transplants.**

METHODS: **We performed a retrospective study of 110 patients with alcohol-associated cirrhosis (77% male; mean age, 55 y; 71% with >6 mo of abstinence) who received liver transplants at a single center in Paris, France, from 2000 through 2013. We collected data on previous exposure to MRFs, steatosis (>10% in the explant), and histologically confirmed hepatocellular carcinoma (HCC).**

RESULTS: **HCC was detected in explants from 29 patients (26%). Steatosis was detected in explants from 47 patients (70% were abstinent for ≥6 mo); 50% had a history of overweight or type 2 diabetes. Fifty-two patients (47%) had a history of MRFs and therefore were at risk for nonalcoholic fatty liver disease. A higher proportion of patients with MRF had HCC than those without MRF (46% vs 9%; $P < .001$). A previous history of overweight or type 2 diabetes significantly increased the risk for HCC (odds ratio, 6.23; 95% confidence interval [CI], 2.47–15.76, and odds ratio, 4.63; 95% CI, 1.87–11.47, respectively; $P < .001$). MRF, but not steatosis, was associated with the development of HCC (odds ratio, 11.76; 95% CI, 2.60–53; $P = .001$) independent of age, sex, amount of alcohol intake, or severity of liver disease.**

CONCLUSIONS: **Patients with alcohol-associated cirrhosis who received transplants frequently also had nonalcoholic fatty liver disease. MRFs, particularly overweight, obesity, and type 2 diabetes, significantly increase the risk of HCC.**

Keywords: Fatty Liver; Alcohol; Metabolic Risk Factors; Hepatocellular Carcinoma; Liver Transplantation.

Despite a period of abstinence that is variable but usually longer than 6 months, some patients receiving a transplant because of alcoholic liver disease (ALD) still have steatosis on the explanted liver. In the general population, a frequent cause of steatosis, unrelated to alcohol consumption, is nonalcoholic fatty liver disease (NAFLD). NAFLD usually is caused by long-term exposure to obesity, diabetes, or different features of the metabolic syndrome, and therefore currently is a very prevalent chronic liver disease. Because both at-risk alcohol consumption and exposure to metabolic risk factors are frequent in the general population, theoretically ALD and NAFLD can co-exist. Some studies notably have shown that this association can result in heightened liver damage.[1] For instance, excessive drinkers who are obese have cirrhosis and alcoholic hepatitis more often than nonobese drinkers.[2] Central obesity and high serum

Abbreviations used in this paper: **ALD, alcoholic liver disease; BMI, body mass index; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LT, liver transplantation; MELD, model for end-stage liver disease; MRF, metabolic risk factors; NAFLD, nonalcoholic fatty liver disease; OLT, orthotopic liver transplantation; OR, odds ratio.**

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glucose level also are associated with more severe fibrosis in a population of patients with ALD.[3] On the other hand, the severity of steatosis has been identified as an independent predictor of fibrosis progression and the development of cirrhosis.[4] Carcinogenesis also might be modulated by the association of these comorbidities. For example, overweight and obesity increase the risk of hepatocellular carcinoma (HCC),[5] whereas diabetes increases both liver cancer incidence and mortality caused by liver cancer.[6] Steatosis also was associated with a higher risk of HCC.[7]

As it becomes increasingly clear that the association between ALD and NAFLD is clinically relevant, a better understanding of the prevalence and prognostic implications of metabolic risk factors (MRFs) (ie, components of the metabolic syndrome such as obesity, diabetes, dyslipidemia, and arterial hypertension) in patients with ALD is useful. In this study we attempted to investigate the prevalence and consequences of steatosis and MRFs in a cohort of patients who underwent liver transplantation for ALD. We hypothesized that the presence of steatosis on the native liver reflects concurrent NAFLD and that patients with steatosis and MRF are at higher risk of developing HCC.

Patients and Methods

This single-center, retrospective study was performed at Pitié-Salpêtrière Hospital (Paris, France). Patients with alcoholic cirrhosis who underwent orthotopic liver transplantation (OLT) from January 2000 to January 2013 were included (Supplementary Methods section).

Past medical history was assessed carefully for previous history of overweight or obesity; maximum body weight before the diagnosis of cirrhosis; as well as for history of type 2 diabetes, currently diagnosed and treated type 2 diabetes; history of arterial hypertension; and dyslipidemia. Clinical and biological data were recorded at the time of listing for liver transplantation (LT), and on the day of OLT. The Child–Pugh score and the model for end-stage liver disease (MELD) score also were calculated.

Because both body mass index (BMI) and glucose regulation are modified in cirrhosis (increased BMI caused by ascites and edema, altered glucose tolerance), MRFs were defined as a history of overweight (BMI >25 kg/m^2 in the past 10–20 y) or type 2 diabetes (clinically diagnosed and treated) based on medical records before the diagnosis of alcoholic cirrhosis; these 2 comorbidities were chosen because they are the most unambiguously associated with insulin resistance among all components of the metabolic syndrome.

Histologic Analysis

All explanted livers were analyzed by a single pathologist. Steatosis was defined as 10% or more hepatocytes containing fat droplets on the pathologic analysis of the explant liver. HCC needed to be confirmed histologically on the explant liver (unless pre-OLT histologic documentation was available in patients who received a down-staging procedure) (Supplementary Methods section).

Statistical Analysis

Patients were compared according to the presence of steatosis ($\geq$10%), metabolic risk factors, and HCC on the explanted liver. Continuous variables with normal distribution were expressed as the mean and standard deviation and compared using the Student *t* test and 1-way analysis of variance. Highly skewed variables were expressed as medians and ranges and compared using the Mann–Whitney *U* test. Categoric variables were compared using the chi-squared test or the Fisher exact test. Univariate analyses were performed to identify possible predictors of HCC such as age, sex, BMI, metabolic risk factors, steatosis, duration and amount of alcohol consumption, abstinence period, Child and MELD scores, and time on the wait list. Logistic regression was used to identify independent predictors of HCC in a multivariate analysis model. The interactions between risk factors were evaluated using the additive model (Supplementary Methods section). The significance level was set at a 2-tailed *P* value less than .05 for all analyses. All analyses were performed using IBM SPSS 21 MacOS statistical software (Chicago, IL) and GraphPad Prism version 6.0 (GraphPad Software Inc, San Diego, CA).

Results

Study Population

A total of 457 OLTs were performed at Pitié Salpêtrière Hospital (Paris, France) between January 2000 and January 2013. After exclusion of causes of chronic liver diseases other than alcohol, 110 patients were included retrospectively (Figure 1). Indications for LT were end-stage liver disease (ie, decompensated cirrhosis) in 76% (N = 84) of patients and HCC developed in ALD in 24% (N = 26). Half of the patients were heavy drinkers (>80 g/d); most of the patients (71%) had a longer than 6-month abstinence period at the time of OLT. Table 1 shows the patient characteristics from the whole cohort and from each of the 2 subgroups according to the initial diagnosis. The pathologic analysis of the native liver did not confirm HCC in 8 cases (without previous down-staging therapy), and incidentally diagnosed HCC tumors in 11 patients. Therefore, 29 patients (26%) were considered to have ALD-related HCC.

As expected, patients listed for end-stage liver disease had more advanced liver disease with higher Child–Pugh and MELD scores than those listed for ALD-related HCC. They also had a shorter waiting period before LT was performed. However, the ALD-related HCC patients

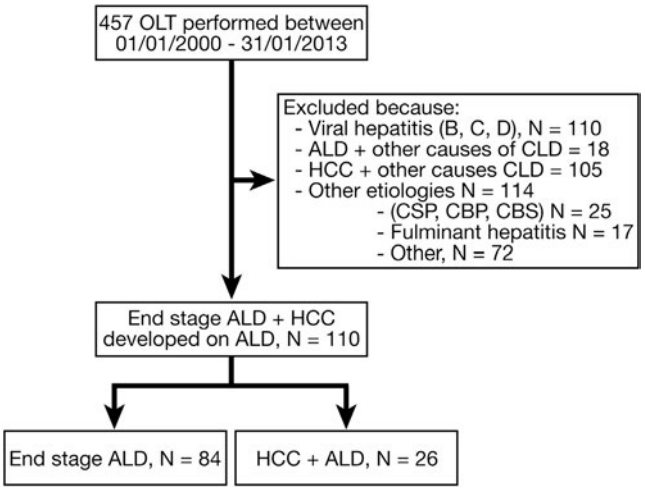

Figure 1. Study flowchart. CBP, primary biliary cirrhosis; CBS, secondary biliary cirrhosis; CLD, chronic liver disease; CSP, primary sclerosing cholangitis.

unexpectedly had a more frequent history of overweight and higher fasting glucose levels than those transplanted for end-stage liver disease.

Steatosis and Metabolic Risk Factors

Steatosis of 10% or higher was present on the explanted liver in 47 patients (43%). Those with steatosis did not differ from those without steatosis for length of abstinence before LT (median, 0.61 y, interquartile range, .4–2.3, vs 1.13 y, interquartile range .08–3.14, respectively, p = .65) or the proportion with alcohol abstinence longer than 6 months (70% vs 73%, respectively; $P = .87$). There were no differences for age, sex, daily alcohol consumption before listing for OLT, and the severity of liver disease measured by Child–Pugh and MELD scores between those with and without steatosis. A history of overweight, diabetes, or arterial hypertension also were equally frequent in both groups; at least one metabolic risk factor was present in 48% vs 47% and BMI as well as serum levels of triglyceride, glucose, total cholesterol, or high-density lipoprotein did not differ significantly (Supplementary Table 1). The results did not change when the comparison was restricted to patients with an abstinence period before LT of longer than 6 months.

A large proportion of patients (33 of 47; 70%) had steatosis in the native liver despite alcohol discontinuation for more than 6 months (median, 28.2 mo; range, 6–80.5 mo). Half of these patients (17 of 33) had a history of overweight or diabetes before listing for OLT, and the remainder (16 of 33) had neither recent alcohol consumption (abstinence period, >6 mo; median, 27.2 mo; range, 7–73 mo) nor metabolic risk factors. Thus, in a large number of cases, persistence of steatosis of the native liver was not caused by continued alcohol consumption but rather by concurrent exposure to features of the metabolic syndrome.

This finding prompted us to investigate the prevalence and determinants of metabolic risk factors in the whole cohort of 110 patients. When strictly defining

Table 1. Patient General Characteristics at Inscription on the Waiting List According to Initial Indication for OLT

		Initial indication for OLT		
	All (N = 110)	Alcoholic ESLD (n = 84)	HCC (n = 26)	*P* value
Age, *y*, mean ± SD	55 ± 7.5	55 ± 7.5	55 ± 8	.7
Sex, % male	77	76	81	.6
Time since the diagnosis of cirrhosis, *y*, median (IQR)	2.5 (3.18)	2.6 (3.2)	2.2 (3.2)	.39
Daily alcohol consumption, *g/d*, median (IQR)	80 (55)	80 (76)	80 (35)	.46
Length of the abstinence period, *y*, median (IQR)	1.15 (2.08)	0.9 (2.19)	1.5 (1.6)	.42
>6 month abstinence, % yes	71	68	85	.09
Child–Pugh score, % A/B/C	23/34/43	14/35/51	54/31/15	<.001
Biological parameters at inscription on the waiting list				
Fasting glucose level, *mmol/L*, median (IQR)	6 (2.65)	6.4 (2.45)	5.1 (2.38)	.03
Total cholesterol level, *mmol/L*, median (IQR)	3.5 (2.16)	3.36 (2.27)	4.12 (2.11)	.14
Triglyceride level, *mmol/L*, median (IQR)	0.74 (0.53)	0.72 (0.50)	0.8 (0.73)	.08
AST level, *IU/L*, median (IQR)	51 (32)	53 (31)	46 (30)	.044
ALT level, *IU/L*, median (IQR)	29 (28)	28 (24)	32 (40)	.23
GGT level, *IU/L*, median (IQR)	90 (117)	67 (109)	121 (100)	.005
Albumin level, *g/L*, median (IQR)	32 (10)	32 (8)	37 (11)	<.001
Prothrombin time, %, median (IQR)	47 (26)	42 (24)	67 (31)	<.001
Serum creatinine, *mmol/L*, median (IQR)	68 (30)	68 (32)	75 (27)	.43
Metabolic risk factors				
Overweight or obesity, %	29	21	54	.001
Type 2 diabetes, %	30	27	39	.3
High blood pressure, %	34	32	39	.5
Dyslipidemia, %	22	24	15	.35
≥1 metabolic risk factors, %	47	42	65	<.04

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ESLD, end stage liver disease; GGT, γ glutamyl transpeptidase; IQR, interquartile range.

Table 2. Patient Characteristics According to the Presence of Metabolic Risk Factors

	MRF (N = 52)	Without MRF (N = 58)	*P* value
Age, *y*, mean ± SD	57.2 ± 5.7	53.5 ± 8.5	<.02
Sex, % male	85	71	.11
Time since the diagnosis of cirrhosis, *y*, median (IQR)	2.9 (3.6)	2.7 (3.4)	.45
Time on the waiting list, *mo*, median (IQR)	1.9 (6.7)	1.5 (3.3)	.37
Daily alcohol consumption, *g/d*, median (IQR)	80 (50)	80 (62)	.93
Length of the abstinence period, *y*, median (IQR)	1.2 (2.6)	0.6 (1.4)	.07
>6 mo abstinence, %	80	64	.06
Child–Pugh score, % A/B/C	28/24/48	17/35/48	.3
MELD score, median (IQR)	18 (7)	20 (14)	.67
Biological parameters at OLT			
Fasting glucose level, *mmol/L*, median (IQR)	6.5 (3.8)	6.8 (3)	.39
Total cholesterol level, *mmol/L*, median (IQR)	3.6 (2.6)	3 (1.6)	.40
Triglyceride level, *mmol/L*, median (IQR)	0.76 (0.52)	0.86 (1.14)	.44
AST level, *IU/L*, median (IQR)	44 (34)	63 (106)	.25
ALT level, *IU/L*, median (IQR)	39 (75)	42 (127)	.23
GGT level, *IU/L*, median (IQR)	122 (221)	45 (101)	.002
Albumin level, *g/L*, median (IQR)	35 (9)	32 (8)	.69
Prothrombin time, %, median (IQR)	45 (25)	45 (31)	.97
Serum creatinine level, *mmol/L*, median (IQR)	80 (57)	68 (22)	.12
Histologic findings, %			
>10% steatosis	42	43	.9
Iron overload	37	40	.7
HCC on the explant liver	46	9	<.001
Metabolic risk factors			
Previous history of overweight, %	62	0	<.001
Type 2 diabetes, %	64	0	<.001
High blood pressure, %	44	24	<.03
Dyslipidemia, %	29	16	.09

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ glutamyl transpeptidase.

metabolic risk factors as a history of overweight and/or diabetes, the prevalence of exposed patients was 47% (52 of 110). Table 2 shows characteristics of those with and without exposure to MRF. A previous history of overweight was present in 62% of patients with MRF, type 2 diabetes was present in 64%, and high blood pressure was present in 44%. Patients with MRF were older, but the amount of daily alcohol consumed, the length of abstinence, and the time on the waiting list did not differ between the 2 groups. Disease severity (Child–Pugh, MELD scores) was similar, and there was no difference in the prevalence of iron overload in patients with or without MRF. However, a striking difference was the higher prevalence of confirmed HCC in patients with MRFs as compared with those without MRF (46% vs 9%; $P < .001$). This finding was confirmed when

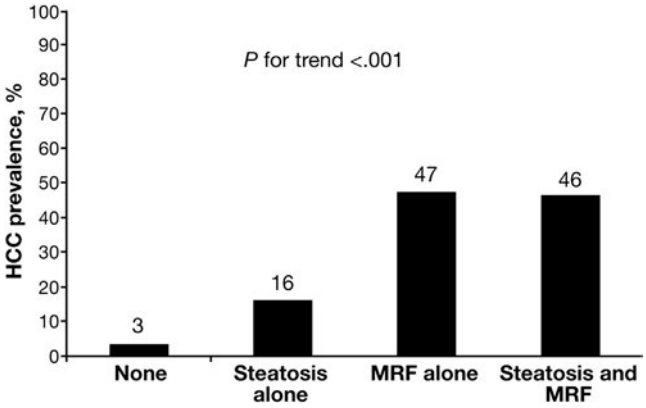

Figure 2. HCC prevalence according to the presence of steatosis and MRF.

MRFs were defined less strictly (to include patients with arterial hypertension and dyslipidemia in addition to those with overweight/obesity or type 2 diabetes) (40% vs 9%; $P < .001$). Figure 2 shows that the proportion of patients with HCC gradually increased between patients showing neither steatosis nor MRFs, those with steatosis only, and those with MRFs.

Hepatocellular Carcinoma in Patients With and Without Metabolic Risk factors

We next analyzed the characteristics and determinants of HCC in more detail. Table 3 shows the main tumor characteristics, as well as patient characteristics according to the presence of HCC. The prevalence of HCC increased with age (34% in patients >55 y vs 17% in patients <55 y; $P < .05$). There was a nonsignificant trend for a lower amount of alcohol consumption and a longer abstinence period in HCC patients. Time on the waiting list until OLT was similar between groups. As expected, HCC patients had more compensated liver disease (46% with Child A cirrhosis).

The prevalence of 10% or more steatosis was similar between groups, and steatosis did not increase the risk of HCC. Interestingly, in this population of excessive drinkers, the amount of daily alcohol consumption was not associated with the risk of developing HCC. However, a striking finding was that 83% of patients with HCC had one or more metabolic risk factors, whereas only 5 of 29 patients ultimately diagnosed with HCC did not have either a history of type 2 diabetes or overweight. A history of diabetes and/or overweight increased the risk of developing HCC by an odds ratio (OR) of 9.1 (95% confidence interval [CI], 3.13–26.40; $P < .001$). Exposure to type 2 diabetes alone increased the HCC risk by an OR of 4.63 (95% CI, 1.87–11.47; $P = .001$) and exposure to overweight by an OR of 6.23 (95% CI, 2.47–15.76; $P = .001$). The synergistic index for the presence of overweight and type 2 diabetes was equal to unit, which indicates an additive effect. Similar results were obtained when exposure to metabolic risk factors was defined less

Table 3. Characteristics of Patients With and Without Histologically Confirmed HCC

	With HCC (N = 29)	Without HCC (N = 81)	*P* value
Age, *y*, mean ± SD	59 ± 7	54 ± 7	.002
Sex, % male	86	74	.18
Time since diagnosis of ALD, *y*, median (IQR)	1.5 (3)	3.1 (3.7)	.26
Time on the waiting list, *mo*, median (IQR)	2.2 (5.1)	1.5 (3.5)	.35
Daily alcohol consumption, *g/d*, median (IQR)	75 (45)	80 (74)	.18
Length of the abstinence period, *y*, median (IQR)	1.2 (1.8)	0.7 (3)	.15
>6 mo abstinence, %	82	68	.15
Child–Pugh score, % A/B/C	46/25/29	14/31/55	<.001
MELD score, median (IQR)	19 (13)	18 (12)	.29
Biological parameters at OLT			
Fasting glucose level, *mmol/L*, median (IQR)	8.4 (5)	6.6 (2.7)	.004
Total cholesterol level, *mmol/L*, median (IQR)	3.8 (3.07)	3 (1.8)	.13
Triglyceride level, *mmol/L*, median (IQR)	0.9 (1.3)	0.7 (0.6)	.06
AST level, *IU/L*, median (IQR)	53 (196)	48 (51)	.68
ALT level, *IU/L*, median (IQR)	62 (162)	61 (86)	.005
GGT level, *IU/L*, median (IQR)	156 (267)	52 (112)	.008
Albumin level, *g/L*, median (IQR)	35 (10)	32 (8)	.04
Prothrombin time, %, median (IQR)	48 (45)	44 (26)	.17
Serum creatinine level, *mmol/L*, median (IQR)	79 (62)	71 (28)	.34
Histologic findings			
>10% steatosis, %	48	41	.48
Iron overload, %	20	33	.14
Metabolic risk factors			
Overweight, %	59	19	<.001
Type 2 diabetes, %	55	21	.001
High blood pressure, %	45	30	.14
Dyslipidemia, %	24	21	.72
≥1 metabolic risk factors, %	83	35	<.001
Tumor characteristics			
Mean number of nodules	4 ± 5	N/A	
Multifocal (>3 nodules) tumor, %	38	N/A	
Mean tumor size	32 ± 18	N/A	
Microvascular invasion, %	31	N/A	
Satellite nodules, %	14	N/A	
Parffit score, mean	3.5 ± 4	N/A	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ glutamyl transpeptidase.

Table 4. Factors Associated With HCC Risk in Multivariate Analysis

Variable	OR (95% CI)	*P*
Age	1.09 (0.98–1.21)	.11
Sex	1.02 (0.16–6.43)	.98
Alcohol consumption, *g/d*	0.98 (0.96–1)	.09
MELD score at OLT	0.98 (0.90–1.10)	.97
Abstinence period	0.99 (0.97–1.01)	.66
MRF[a]	11.76 (2.60–53)	.001

[a]Overweight, obesity, or type 2 diabetes.

stringently as exposure to either overweight or type 2 diabetes, or arterial hypertension together with dyslipidemia (OR, 6; 95% CI, 2.08–17.29; $P = .001$).

Exposure to metabolic risk factors was the strongest risk factor of HCC in a model adjusted for age, sex, severity of liver disease (MELD score), alcohol consumption, and abstinence period (Table 4). In the same model, the effect magnitude was greater for previous history of overweight (OR, 9.06; 95% CI, 2.17–37.78; $P = .002$) than for type 2 diabetes (OR, 6.10; 95% CI, 1.56–23.85; $P = .009$). Results were similar in a model that tested hepatic iron overload and the amount of hepatic steatosis (Supplementary Tables 2 and 3).

Discussion

The main finding of this study was that patients with advanced ALD have a high prevalence of NAFLD, and that this comorbid association confers a significantly increased risk of hepatocellular carcinoma.

The population described in this study represents the most severe end of the spectrum of alcoholic liver disease. All patients had cirrhosis and were listed for liver transplantation, either for end-stage liver disease or for hepatocellular carcinoma. They were almost all from a homogenous Caucasian background and had a massive, lifetime exposure to excessive alcohol intake. Despite this seemingly typical clinical picture of severe alcoholic liver disease, a striking finding of this study was the high prevalence of metabolic risk factors together with steatosis, which strongly suggests that these patients also had NAFLD. Almost half of the patients listed for end-stage ALD had long-term exposure to the cardiometabolic risk factors of NAFLD. More than two thirds of patients who had stopped consuming alcohol for longer than 6 months before they were listed for liver transplantation had significant steatosis on the native liver by the time of OLT; a sizeable fraction of these patients had long-standing overweight, obesity, or type 2 diabetes. Patients with steatosis did not have a higher daily alcohol consumption in the immediate past. Moreover, a 3- to 6-month abstinence period should clear hepatic steatosis because liver fat is highly labile.[8] It therefore is improbable that persistent steatosis in these patients with metabolic features is caused by alcohol consumption. Hence, this series provides indirect evidence that NAFLD and ALD can co-exist owing, at least in part, to the epidemiologic interaction between 2 frequent disorders. Thus, patients with ALD need to be screened for NAFLD and its associated comorbidities.

When comparing patients with or without steatosis of the native liver, there were no obvious differences in disease severity for either hepatic insufficiency or HCC in this selected population with very advanced ALD. This

suggests that steatosis per se is a marker of exposure to cardiometabolic risk factors and NAFLD, but is not responsible for a worse disease course. In contrast, patients carrying metabolic risk factors, mainly overweight/obesity or diabetes, had a 9-fold higher risk of hepatocellular carcinoma. This increased risk was independent of age and sex, 2 strong factors that are associated with hepatic carcinogenesis. It also was independent of the amount of alcohol consumed, despite a well-described linear increase in HCC risk in excessive drinkers.[9] It is striking that of the 29 patients ultimately diagnosed with histologically proven HCC, only 5 (17%) did not have a history of overweight/obesity or type 2 diabetes. These data highlight the carcinogenic potential of the main features of the metabolic syndrome when present in patients with ALD. Our interpretation is that superimposed NAFLD is seen frequently in end-stage ALD, and that it adversely impacts prognosis, particularly by increasing the risk of HCC. Previous studies have shown that excessive drinkers who are obese have a higher risk of developing cirrhosis,[2] and that a high serum glucose level is associated independently with increased fibrosis.[10] Because the present study included only patients with alcoholic cirrhosis listed for transplantation, we could not assess a potentially deleterious effect of superimposed NAFLD on fibrogenesis or on hepatic insufficiency.

A recent report from northern Europe has stirred controversy by reporting a much lower than expected risk of HCC in patients with ALD, with a low impact on overall mortality.[11] In a nationwide Danish cohort of 8482 newly diagnosed cirrhotic patients, the risk of HCC was only 1% (95% CI, 0.8%–1.3%), with an annual risk ranging from 0.25% to 0.5%.[11] These estimates are much lower than the recommended threshold of 1.5% per year defining cost effectiveness for HCC screening according to guidelines from scientific societies.[12] Other population-based studies from northern Europe have reported low rates of HCC in ALD patients, with one Scandinavian study showing a 6-fold lower HCC risk after a 15-year follow-up period in alcoholic cirrhosis compared with viral cirrhosis.[13] In contrast, studies from southern Europe have reported higher HCC incidence rates, which raised the question of whether these differences might be related to different epidemiologic designs (ie, community-based vs clinic-based). Sherman[14] concluded that "the risk of HCC in ALD is variable and should be assessed locally." Based on data presented here, we suggest the additional explanation that superimposed NAFLD and associated overweight/obesity or type 2 diabetes could increase the neoplastic risk in patients with alcoholic cirrhosis. Similar to our study, Jepsen et al[11] did find that male sex and increasing age were risk factors of HCC, but they could not adjust for obesity and diabetes in their cohort of alcoholic cirrhotic patients. Both obesity and diabetes are recognized risk factors of HCC at the population level.[15] Hence, some of the differences in HCC risk between different ALD cohorts can reflect differences in exposure to factors predisposing to NAFLD. These findings are important because they might help identify a subpopulation of patients with alcoholic cirrhosis at higher risk of HCC who thus are amenable to cost-effective HCC screening.

The current series extends to a population with ALD with heavy alcohol intake data showing that obesity and diabetes are bona fide HCC risk factors. A large, multinational European cohort study has shown that the population-attributable fraction of HCC related to obesity was 16.1% vs 13.2% for hepatitis B virus (HBV) infection, 10.2% for alcohol consumption, and 20.9% for hepatitis C virus infection.[16] These data were corroborated in a US population-based study of individuals older than age 65 years in whom the population-attributable factor for obesity/diabetes was 36.6%, the highest of all examined risk factors, including hepatitis viral infection.[17] In a very large US health care claims database, NAFLD and type 2 diabetes were the top underlying risk factors for HCC.[18] Finally, the metabolic syndrome, as well as its individual components, were associated independently with an increased risk of HCC in both US[19] and European populations.[15] It is important to note that although diabetes is an ongoing risk factor, major ponderal changes might occur because of advanced cirrhosis. Some of the patients with a history of overweight/obesity included here might no longer be overweight at listing for transplantation. However, obesity without diabetes was a strong factor associated with HCC. We can only speculate for the reasons of this apparent discrepancy. Long-standing exposure to excess body weight could act as a tumor initiation step favoring clonal emergence that will develop into full-blown clinical neoplasms only years later, when significant changes in body weight might have occurred. Tumors with a slow growth before clinical diagnosis or imaging characterization therefore might be diagnosed when excessive adiposity is no longer present.

Some studies have reported a synergistic effect between alcohol consumption and diabetes[20] or obesity[21] for the risk of HCC, whereas one study showed that obesity was an independent risk factor for HCC in alcoholic cirrhosis.[22] The current study extends these findings to patients with the most severe forms of alcoholic liver disease requiring liver transplantation. Even in heavy drinkers with severe alcoholic cirrhosis, the impact of MRF and concurrent NAFLD is not negligible and may precipitate the occurrence of HCC. Interestingly, the detrimental role of obesity and diabetes in HCC occurrence also has been shown in patients with hepatitis C virus[23] or HBV chronic hepatitis,[24] including those living in highly endemic areas for viral hepatitis such as the Far East for HBV.[24]

Our study had several limitations. Data collection was retrospective and performed at a single center. The assessment of some of the metabolic risk factors such as overweight was based on personal history and possibly could have been influenced by recall bias. Nonetheless,

most of the information was cross-checked with data available in the medical records, before the diagnosis of decompensated cirrhosis. The ascertainment of diabetes was less problematic because it was based either on a documented increase in serum glucose or glycosylated hemoglobin level before the diagnosis of cirrhosis, or on regular intake of antidiabetic medications. Other components of the metabolic syndrome such as hypertension and dyslipidemia could have been influenced by the cirrhotic state; if no data were available before the diagnosis of cirrhosis, these conditions could have been underdiagnosed. However, these limitations when studying exposure to metabolic risk factors were not specific to our study. Abstinence was documented through anamnesis, based on patient reports or on close relatives, or through regular psychiatric assessments throughout the pretransplant work-up. However, there was no systematic monitoring protocol of alcohol blood levels and therefore minor or episodic alcohol consumption could not be ruled out. Consequently, the distinction between alcohol-related and metabolically driven steatosis could have been, at times, less reliable. We believe that, overall, these limitations did not alter the main conclusion of this study. Another important limitation of our study was the small sample size of highly selected patients with end-stage alcoholic liver disease. Further studies including patients with the whole spectrum of alcoholic liver disease should be performed to confirm our findings and analyze the magnitude of the interaction between alcohol consumption and metabolic risk factors on fibrosis progression, development of cirrhosis, and hepatocellular carcinoma.

In conclusion, heavy drinkers with alcoholic cirrhosis listed for liver transplantation frequently have concurrent metabolic risk factors such as overweight/obesity and type 2 diabetes, together with other components of the metabolic syndrome, as well as steatosis, despite cessation of alcohol intake. This strongly argues for the co-existence of ALD and NAFLD, a morbid association that needs to be diagnosed because it significantly increases the risk of developing HCC. Despite a reported overall lower incidence of HCC in alcoholic cirrhosis, the present study shows that the segment of patients with concurrent NAFLD may be at much higher risk of HCC than the remainder of the population with alcoholic cirrhosis. These findings are important for risk stratification of HCC in patients with ALD, and further studies are needed to confirm the cost effectiveness of screening for HCC in this target population.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2014.10.011.

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Supplementary Methods

Patients with concurrent causes of chronic liver disease, such as viral hepatitis, autoimmune liver disease, primary biliary cirrhosis, sclerosing cholangitis, Wilson's disease, polycystic liver disease, or cryptogenic cirrhosis, were excluded. In addition, patients with fulminant hepatitis of other etiologies than alcohol were excluded.

The diagnosis of alcoholic cirrhosis was considered if self-reported alcohol consumption exceeded 80 g/d for more than 10 years consecutively. Past daily alcohol consumption (calculated as an average consumption in the few years before the diagnosis of cirrhosis) and the abstinence period before being listed on the waiting list were recorded based on individual self-reporting corroborated by the patient's transplant hepatologist and psychiatric evaluation reports before OLT. In our center, patients with alcoholic cirrhosis had to be abstinent for 6 months or more to be listed for OLT. However, in a few patients, the 6-month abstinence period was waived if they had a severe first episode of acute alcoholic hepatitis, no serious comorbidities, supportive family members, and a commitment to alcohol abstinence (based on careful psychiatric evaluation).

Histologic Analysis

For patients with HCC, tumor characteristics, including size, number of nodules, presence of capsule, microvascular and macrovascular invasion, presence and number of satellite nodules, and presence of necrosis or hemorrhage were reported. The Parfitt's pathologic score on the explanted liver, a measurement associated with HCC recurrence after OLT, was calculated for each case.

Statistical Analysis

The synergy index was calculated as follows: synergy index $= [OR_{A+B+} - 1]/[OR_{A+} - 1] + [OR_{B+} - 1]$, with OR_{A+B+} indicating the presence of joint risk factors, and OR_{A+} and OR_{B+} indicating the presence of one risk factor in the absence of the other. A synergy index value equal to 1 was considered indicative of additivity, and a value >1 was indicative of superadditivity or synergy.

Supplementary Table 1. Patient Characteristics According to the Presence of More Than 10% Steatosis

	With steatosis (N = 47)	Without steatosis (N = 63)	*P* value
Age, *y*, mean ± SD	55 ± 8	55 ± 7	.9
Sex, % male	79	76	.8
Time since the diagnosis of cirrhosis, *y*, median (IQR)	2.8 (2.8)	2.26 (3.2)	.87
Time on the waiting list, *mo*, median (IQR)	1.7 (7.4)	2.8 (3.8)	.81
Daily alcohol consumption, *g/d*, median (IQR)	87 (50)	80 (58)	.51
Length of the abstinence period, *y*, median (IQR)	1.2 (2.7)	1.1 (1.9)	.65
>6 mo abstinence, %	70	73	.8
Child–Pugh score, % A/B/C	28/33/39	18/27/55	.3
MELD score, median (IQR)	18 (10)	18 (10)	.43
Biological parameters at OLT			
Fasting glucose level, *mmol/L*, median (IQR)	6.9 (3.2)	6.5 (3.2)	.38
Total cholesterol level, *mmol/L*, median (IQR)	3.6 (1.8)	3 (2.11)	.57
Triglyceride level, *mmol/L*, median (IQR)	0.97 (1.04)	0.66 (0.6)	.024
AST level, *IU/L*, median (IQR)	59 (100)	46 (54)	.32
ALT level, *IU/L*, median (IQR)	43 (61)	40 (117)	.65
GGT level, *IU/L*, median (IQR)	91 (143)	79 (166)	.56
Albumin level, *g/L*, median (IQR)	34 (5)	33 (10)	.72
Prothrombin time, %, median (IQR)	47 (33)	44 (27)	.34
Serum creatinine level, *mmol/L*, median (IQR)	70 (33)	76 (30)	.52
Histologic findings			
Iron overload, %	32	44	.2
HCC on the explant liver, %	30	24	.5
Metabolic risk factors			
Overweight, %	34	25	.3
Type 2 diabetes, %	23	35	.2
High blood pressure, %	34	33	.9
Dyslipidemia, %	23	21	.7
≥1 metabolic risk factors, %	47	48	.9

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ glutamyl transpeptidase.

Supplementary Table 2. Independent Predictors of HCC in Multivariate Analysis

	Model 1		Model 2	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.08 (0.97–1.12)	.16	1.10 (0.99–1.22)	.07
Sex	1.23 (0.24–6.26)	.81	1.08 (0.21–5.52)	.93
Alcohol consumption, *g/d*	0.98 (0.97–1.00)	.07	0.99 (0.97–1.01)	.19
MELD score at OLT	1 (0.91–1.09)	.94	0.99 (0.91–1.09)	.88
Overweight/obesity	8.24 (2.04–33.32)	.003	–	–
Type 2 diabetes	–	–	5.59 (1.53–20.49)	<.01

NOTE. When considered separately, overweight/obesity (model 1) and type 2 diabetes (model 2) were predictors of HCC risk independent of classic factors (age, sex, alcohol consumption, and severity of liver disease expressed as MELD score).

Supplementary Table 3. Independent Predictors of HCC in Multivariate Analysis in a Model Including Hepatic Steatosis and Iron Overload

	OR (95% CI)	*P*
Age	1.12 (1.00–1.26)	<.06
Sex	0.73 (0.10–5.43)	.76
Alcohol consumption, *g/d*	0.99 (0.97–1.01)	.32
MELD score at OLT	1.03 (0.93–1.14)	.63
Steatosis, %	1.03 (0.99–1.08)	.17
Iron overload	0.54 (0.21–1.38)	.17
MRFs[a]	13.85 (2.72–70.42)	.002

[a]When the amount of hepatic steatosis and iron overload were added to the model, MRFs (overweight/obesity and type 2 diabetes) still were independent predictors of HCC risk.

VI -2. Résumé de l'article 2.

VI-2.1.Méthodes.

Nous avons inclus les patients ayant subi une transplantation hépatique pour cirrhose alcoolique à l 'Hôpital Pitié-Salpetrière dans la période janvier 2000 – janvier 2013. Le diagnostic de cirrhose alcoolique a été porté si la consommation d 'alcool était de > 80 g/jour pour une durée supérieure ou égale à 10 ans.

La quantité moyenne d'alcool par jour, la durée de la consommation ainsi que la durée de sevrage ont été recueillies des dossiers médicaux (interrogatoire des patients ou des membres de la famille, évaluation par l'hépatologue et l'addictologue).

Les données cliniques et biologiques ont été recueillies au moment de l'inscription sur la liste d'attente et le jour de la greffe. Les antécédents de surpoids ou obésité, de diabète type 2, d'hypertension artérielle et de dyslipidémie ont été recueillis des dossiers médicaux des patients avant le diagnostic de cirrhose ou avant le premier épisode de décompensation. Les facteurs de risque métabolique ont été définis par la présence soit d'un surpoids ou d'une obésité soit d'un diabète type 2.

L'analyse histologique du foie explanté a été réalisée par un seul anatomopathologiste. La présence d'une stéatose était retenue si elle était > 10%. Le diagnostic de carcinome hépatocellulaire a été retenu si : (1) confirmation sur l'explant ou (2) documentation histologique avant la greffe pour les patients ayant bénéficié d'une traitement antérieur. La taille et le nombre des nodules, l'envahissement micro- et macrovasculaire et les nodules satellites, ont été également recueillis.

VI-2.2. Résultats principaux et discussion

457 patients ont été transplantés du foie dans la période de l'étude, dont 110 avec une cirrhose alcoolique (y compris 26 avec carcinome hépatocellulaire développé sur cirrhose alcoolique). Apres l'analyse de l'explant, le diagnostic de carcinome hépatocellulaire a été confirmé chez 29 patients (26%), dont une découverte fortuite sur explant chez 11 patients. La moitié des patients consommaient > 80 g alcool/j avant la greffe ; 71% des patients avaient une durée de sevrage de > 6 mois au moment de la greffe.

Nous avons analysé dans un premier temps la prévalence de la stéatose et des facteurs de risque métabolique.

La stéatose de > 10% était présente chez 43% des patients. La prévalence des facteurs de risque métabolique, la consommation d'alcool et la durée de sevrage étaient similaires parmi les patients avec ou sans stéatose. Parmi les patients avec stéatose, 70% avaient une durée de sevrage de > 6 mois ; parmi ces derniers, 50% avaient des antécédents d'obésité ou de diabète.

Compte tenu du fait que la stéatose est rapidement réversible avec le sevrage et que 2/3 des patients avaient plus de 6 mois de sevrage, il est peu probable que la persistance de la stéatose soit due à la consommation d'alcool. Il apparait plus probable que l'exposition aux facteurs de risque métabolique soit la raison principale expliquant la présence de la stéatose. Ceci permet donc de reconnaitre la coexistence possible de la NAFLD chez les patients ayant une MAF même à un stade avancé de cirrhose. Les scores de gravité de la cirrhose (Child Pugh et MELD) et la prévalence du carcinome hépatocellulaire étaient similaires chez les patients avec et sans stéatose. Il est donc peu

probable que la stéatose en soi soit pathogène mais celle-ci représente davantage un marqueur d'exposition aux facteurs de risque métabolique

Les lésions histologiques de la MAF et de la NAFLD présentent de nombreuses similarités rendant la distinction entre ces deux conditions pratiquement impossible sur l'histologie seule.

Ces deux étiologies de stéatose partagent des mécanismes communs. Lors de la consommation d'alcool, la production d'acetaldéhyde entraine une altération de l'export des triglycérides par la voie des VLDL résultant en une stéatose et la formation de radicaux libres par peroxydation lipidique ce qui favorise des lésions de nécrose hépatocytaire[326, 327]. Dans l'obésité l'augmentation du flux d'acides gras libres libérés à partir du tissu adipeux vers le foie, favorise la stéatose. L'altération de la beta-oxydation mitochondriale et le déséquilibre entre la synthèse et l'export des triglycérides favorise l'accumulation des métabolites toxiques d'acides gras libres responsables de la lipotoxicité et l'apparition des lésions hepatocytaires[1].

47% des patients dans notre cohorte avaient un ou plusieurs facteurs de risque métabolique, dont l'obésité ou le diabète de type 2. La durée de la maladie, la consommation d'alcool, la période d'abstinence et les scores de Child-Pugh et MELD étaient similaires chez les patients avec ou sans facteurs de risque métabolique. En revanche, la prévalence du carcinome hépatocellulaire augmentait progressivement de 3% chez les patients sans stéatose ni facteurs de risque métabolique à 16% chez les patients avec stéatose et 46% chez les patients cumulant les deux ($p < 0.001$). 83% des patients avec carcinome hépatocellulaire avaient un ou plusieurs facteurs de risque métabolique. Nous avons démontré que l'exposition aux facteurs de risque métabolique était un facteur de risque indépendant pour le carcinome hépatocellulaire après ajustement pour l'âge, le sexe, la consommation d'alcool et la durée du sevrage (OR =

11.76, 95% CI 2.60 – 53, p < 0.001). En revanche, la stéatose n'était pas associée avec une augmentation indépendante du risque de carcinome hépatocellulaire.

Des études antérieures suggèrent qu'il existe une relation linéaire dose-dépendante entre la quantité d'alcool et le risque de développer un carcinome hépatocellulaire, avec un risque significativement plus élevé pour une consommation moyenne dépassant 75 g/jour[328].

D'autres études suggèrent qu'au delà d'une valeur seuil, le risque de développer une cirrhose et ses complications n'est plus dose dépendant[329]. Alors que la plupart des patients dans notre cohorte avaient une consommation excessive d'alcool (>80 g/jour chez 50% des patients), l'augmentation du risque de carcinome hépatocellulaire était indépendante de la quantité ou la durée de la consommation d'alcool. Ces résultats laissent penser que l'effet de l'alcool est plutôt permissif et que d'autres facteurs interviennent probablement dans le développement des complications liées à l'alcool.

Nous avons trouvé une prévalence élevée des facteurs de risque métabolique chez les patients avec carcinome hépatocellulaire dans cette cohorte (83%). Le syndrome métabolique ainsi que ses composantes, l'obésité et le diabète, sont associés à une augmentation du risque de carcinome hépatocellulaire dans la population générale en Europe et aux Etats-Unis[174, 330].

Dans une large étude Européenne, la fraction du risque attribuable à l'obésité et l'alcool était de 16% et 10%, respectivement, ce qui était du même ordre de grandeur que celle de l'hépatite B ou C (13% et 21% respectivement)[331].

Dans une autre étude américaine, la NAFLD et le diabète étaient identifiés parmi les causes les plus fréquentes de carcinome hépatocellulaire chez 58% et respectivement 36% des patients[208].

Dans un large étude réalisée dans la population générale au Taiwan, Chen et al. ont démontré un effet synergique de l'obésité ou du diabète et du portage des virus B et C, responsable d'une augmentation significative du risque de carcinome hépatocellulaire. En l'absence d'infection virale B ou C l'augmentation du risque de CHC était proportionelle au nombre des composantes du syndrome métabolique [202].

L'interaction entre la consommation d'alcool et les facteurs de risque métabolique et leur impact sur la carcinogenèse hépatique a été étudiée dans différentes études.

Une analyse du registre américain de transplantation hépatique a démontré que le risque de carcinome hépatocellulaire était trois fois plus élevé chez les patients obèses avec cirrhose alcoolique[185]. Inversement, la consommation d'alcool, même modérée, augmentait significativement le risque de carcinome hépatocellulaire chez les patients avec NAFLD[205].

D'autres études ont mis en évidence un effet synergique entre la consommation excessive d'alcool et l'obésité ou le diabète[186-188] pour le risque de CHC.

Dans notre étude l'obésité a été associée avec une augmentation plus importante du risque de développer un carcinome hépatocellulaire (OR = 6.23, 95% CI 2.47 – 15.76, p = 0.001) que le diabète (OR = 4.63, 95% CI 1.87 – 11.47, p = 0.001). L'effet additif observé du diabète et de l'obésité (index de synergie = 1) suggère des mécanismes carcinogéniques communs pour ces deux conditions : l'hyperinsulinémie, les cytokines pro-inflammatoires, le stress oxydatif et la lipotoxicité, responsables de l'activation des voies oncogéniques[332]. Cliniquement, aux stades avancés de la cirrhose, l'obésité ou le surpoids peuvent avoir disparu. Il est dès lors important de rechercher à l'interrogatoire une exposition passée à l'excès pondéral ou aux autres facteurs métaboliques afin de pouvoir établir si le patient encourt un risque supplémentaire.

Les principales limites de notre étude sont : (1) le recueil rétrospectif des données et le nombre limité de sujets ; (2) la difficulté d'identifier les facteurs de risque métaboliques chez les malades avec cirrhose décompensée ; (3) l'impossibilité de généraliser les résultats pour la MAF non-cirrhotique.

Malgré ces limites, ce travail démontre que l'exposition passée aux facteurs de risque métabolique augmente significativement le risque de carcinome hépatocellulaire chez les patients avec une MAF au stade de cirrhose et pourrait être un outil important pour identifier les sujets à risque.

Chapitre VII. ***Article 3.***

"Fatty Liver Is An Independent Predictor Of Early Carotid Atherosclerosis: Results From a Large Transversal Study and Long-Term Follow-Up"

VII-1. Rationnel et Objectifs du Travail

La maladie cardiovasculaire est une des causes la plus fréquente de décès dans le monde (75% des décès dus aux maladies cérébrovasculaires ou infarctus du myocarde). Pour ces raisons, il est important d'identifier les facteurs de risque permettant d'agir en prévention primaire.

La mesure de l'épaisseur intima-media carotidienne (EIMc) est une marqueur précoce d'athérosclérose avec une valeur prédictive reconnue pour les évènements cardio-vasculaire futures en plus des facteurs de risque classiques [277].

L'athérosclérose et la NAFLD ont de nombreux facteurs de risque communs, à travers le syndrome métabolique[313, 333].

L'état actuel de connaissances concernant l'association entre la NAFLD, l'EIMc et le risque CV a été présenté en détail dans la partie générale de ce manuscrit (***voir chapitre IV***).

En résumé, les données actuelles montrent incontestablement une association entre la NAFLD et l'augmentation de la prévalence et l'incidence des lésions précoces d'athérosclérose, des évènements et de la mortalité CV[164, 168, 257, 298, 334, 335].

En raison des limites méthodologiques et l'hétérogénéité des études antérieures, certains aspects concernant l'association entre la NAFLD et la maladie CV doivent encore être clarifiés et méritent d'être explorés. Certes, il existe des liens étroits entre la NAFLD

et le syndrome métabolique qui est un facteur de risque CV connu, ce qui pourrait expliquer au moins en partie l'association fréquente entre la NAFLD et la maladie CV. D'une part, le syndrome métabolique favorise la stéatose, d'autre part la stéatose est un facteur de risque pour le développement du syndrome métabolique[336]. Des études de suivi à long terme ont montré que le syndrome métabolique accélère la progression des lésions d'athérosclérose[337]. Néanmoins, si la NAFLD est un facteur de risque CV au delà de l'association avec le syndrome métabolique reste un sujet de controverse. Surtout, il n'existe pas à présent des études longitudinales pour déterminer si la présence de la NAFLD en plus du syndrome métabolique accélère la progression des lésions d'athérosclérose. Une question importante serait de savoir si la progression ou la régression de la NAFLD influence l'évolution des lésions d'athérosclérose et modifie en conséquent le risque CV.

Les objectifs de notre étude étaient de : (1) analyser dans une population avec deux ou plusieurs facteurs de risque CV la relation entre la NAFLD et les valeurs de l'EIMc, la prévalence des plaques carotidiennes ainsi que le score de Framingham; (2) analyser l'impact de la NAFLD sur l'évolution des lésions d'athérosclérose à long terme (progression ou régression de l'EIMc, apparition/disparition des plaques carotidiennes, risque CV).

Article 3. **Fatty Liver Is An Independent Predictor Of Early Carotid Atherosclerosis: Results From a Large Transversal Study and Long-Term Follow-Up**

Fatty Liver as an Independent Predictor of Early Carotid Atherosclerosis: Results From a Large Transversal Study and Long-Term Follow-Up

Short running title: Fatty Liver and carotid atherosclerosis

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Abstract

Whether steatosis is incidentally or causally associated with carotid atherosclerosis is debated and long-term follow-up data are missing.

Aim: to examine the impact of steatosis on the presence and progression of cartotid intima-media thickness (C-IMT) and carotid plaques (CP) in a large cohort with longitudinal follow-up.

Method: Retrospective single-center study between 1995 and 2012. Transversal cohort: patients with ≥2 cardiovascular risk factors without previous cardiovascular events. Longitudinal cohort: patients with two consecutive C-IMT measurements more than 2 years apart. Steatosis was defined by a surrogate marker, the Fatty Liver Index (FLI). CP and C-IMT were assessed by carotid ultrasound.

Results: In the transversal cohort (n=5671) both C-IMT and the Framingham Risk Score (FRS) increased across FLI quartiles (0.58±0.12, 0.61±0.14, 0.63 ±0.14, 0.64±0.14 mm, and 5±5%, 9±7%, 12±8%, 15±9%, p<0.001 for both). Steatosis predicted C-IMT better than diabetes or dyslipidemia. Steatosis independently predicted C-IMT (p=0.002) and FRS (p<0.001) after adjustment for metabolic syndrome and cardiovascular risk factors.

In the longitudinal cohort (n=1872, mean follow-up 8±4 years), steatosis occurred in 12% and CP in 23% of patients. C-IMT increased in patients with steatosis occurrence (from 0.60±0.13mm to 0.66±0.14mm, p=0.001) whereas it did not change in those that stayed free of steatosis. Steatosis at baseline predicted CP occurrence (OR=1.63, 95% CI 1.10–2.41, p=0.014), independent of age, sex, type-2 diabetes, tobacco use, hsCRP, hypertension and C-IMT.

Conclusion: In patients with metabolic syndrome at risk for cardiovascular events, steatosis contributes to early atherosclerosis and progression thereof, independent of traditional cardiovascular risk factors.

Keywords: Fatty liver, atherosclerosis, carotid intima-media thickness, carotid plaques, cardiovascular risk, metabolic syndrome, type 2 diabetes.

List of Abbreviations:

C-IMT: carotid intima-media thickness; FLI: fatty liver index; NAFLD: non alcoholic fatty liver disease; NASH: non alcoholic steatohepatitis; FRS : 10 year Framingham risk score.

Non-alcoholic fatty liver disease (NAFLD) is an increasingly common condition seen in patients with obesity, type 2 diabetes, atherogenic dyslipidemia and arterial hypertension. The leading cause of death in patients with NAFLD is cardiovascular mortality, which is not surprising given the high prevalence of the above-mentioned cardiometabolic risk factors[1, 2]. However, a large body of data indicates that the fatty and inflamed liver expresses several pro-inflammatory and procoagulant factors, as well as genes involved in accelerated atherogenesis[3, 4]. This raises the possibility that the link between NAFLD and cardiovascular mortality might not simply be mediated by shared, underlying, common risk factors but rather that NAFLD independently contributes to increasing this risk.

While an increased prevalence of cardiovascular disease in NAFLD is largely accepted, existing data also show an increased incidence[5-7]. This suggests that steatosis predates clinical cardiovascular disease, and that it may trigger or accelerate its occurrence. Providing support for this causal hypothesis, some reports have demonstrated an increased proportion of subclinical atherosclerosis or pre-atherosclerotic lesions in patients with NAFLD. For instance, ultrasound-diagnosed steatosis was associated with increased coronary calcium scores[8] and with increased intima-media thickness (C-IMT)[9], independent of conventional cardiovascular risk factors and insulin resistance. C-IMT is a marker of early atherosclerosis that predicts coronary and cerebrovascular events: a 0.1 mm increase in C-IMT increases the risk of myocardial infarction by 10–15% and the risk of stroke by 13–18%[10]. Taken together, these data suggest that steatosis actively contributes to atherogenesis. However, there are few, if any, longitudinal, long-term studies assessing the impact of steatosis on the progression of pre-atherosclerotic lesions. In this study we hypothesized that steatosis is an independent predictor of C-IMT progression. Our objectives were: (1) to determine the relationship between steatosis, C-IMT and the 10-year Framingham risk score (FRS) in a population at-risk for cardiovascular events; and (2) to determine in a longitudinal follow-up study if the occurrence or reversal of steatosis independently predicts the occurrence of carotid plaques.

MATERIALS AND METHODS

Study population

The initial study population included individuals between 20 and 75 years of age, seen between 1995 and 2012 in a Primary Cardiovascular Prevention Center, at Pitié-Salpêtrière Hospital, Paris, France. Inclusion criteria were: (1) at least two cardiovascular risk factors among the following: age > 60 years in women and > 50 years in men; systolic blood pressure ≥130 or diastolic blood pressure ≥ 85 mmg Hg or treatment of previously diagnosed hypertension; fasting plasma glucose ≥5.6 mmol/l or previously diagnosed type 2 diabetes; triglycerides levels ≥1.7 mmol/l or HDL <1.03 mmol/l in males or <1.29 mmol/l in females or specific treatment for lipid disorders; overweight (BMI >25 kg/m^2); tobacco consumption; and (2) available carotid ultrasound with measurement of carotid intima-media thickness and of carotid plaques. Exclusion criteria were: patients with previous history of cardiovascular events (myocardial infarction, coronary by-pass surgery or coronary angioplasty, stroke); excessive alcohol consumption; any other identified causes of chronic liver disease; positive test for human immunodeficiency virus (HIV); active malignancy; solid organ or bone marrow transplant recipients. Finally, 5671 patients met the inclusion and exclusion criteria (transversal cohort); among these, 1872 patients had a follow-up carotid ultrasound performed at least two years after the initial evaluation (longitudinal cohort).

Clinical and Laboratory Evaluation

Clinical data were recorded for each patient: age, gender, smoking status, alcohol consumption, (based on self reported frequency and the amount of daily consumption), past medical history; systolic and diastolic blood pressure and anthropometric parameters were measured on the day of hospital visit. Fasting blood samples were collected the day of medical visit. Serum ALT levels were classified as follows: (1) normal-low ALT: <19 IU/l in women and <30 IU/l in men; (2) normal-high ALT: between 19 IU/l and 40 IU/l in women and 30 IU/l and 40 IU/l in men; (3) high ALT: >40 IU/l both in men and in women[11]. Metabolic syndrome was defined according to the IDF criteria[12].

Diagnosis of steatosis. A well validated, surrogate marker, the Fatty Liver Index (FLI) was used to identify patients with steatosis [13]. FLI was calculated as follows:

FLI = ($e^{0.953*\log_e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log_e(\text{GGT}) + 0.053*\text{waist circumference} - 15.745}$) / ($1 + e^{0.953*\log_e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log_e(\text{GGT}) + 0.053*\text{waist circumference} - 15.745}$) * 100. In accordance with the original report and subsequent validation studies steatosis was defined as FLI ≥60. Steatosis occurrence during follow-up was defined as transition from a FLI <60 at baseline to a FLI ≥60 at the end of follow-up.

Evaluation of pre-atherosclerotic lesions and of cardio-vascular risk score.

Carotid ultrasound was performed systematically in all patients as part of a primary prevention program. The C-IMT was measured on the far wall of the carotid artery as the distance between the lumen-intima interface and the media-adventitia using high resolution B-mode ultrasound (Sequoia 512, Acuson). All measurements of C-IMT were made at a site free of any plaque with the accuracy of the electronic caliper to the nearest 0.1 mm. The presence of plaques was defined as localized echo structures encroaching into the vessel lumen for which the distance between the media-adventitia interface and the internal side of the lesion was >1 mm. When a plaque was present, optimal frozen images (1 longitudinal and 1 transversal view), showing the plaque in its greatest thickness, were selected, measured and stored.

All measurements were done by two trained physicians who had more than 10 years experience and 5000 examinations performed[14, 15]. The interobserver coefficient of variation for C-IMT was < 3%.

The 10-Year FRS was calculated using gender specific score sheets.

Statistical methods

All quantitative data were expressed as mean ± standard deviation (SD); categorical data were expressed as percentage. To avoid colinearity, we ensured that variables used in FLI calculation were not included in FRS formula.

Transversal study. The differences in patient's characteristics according to the presence of fatty liver (defined as FLI ≥ 60) were assessed using either Student T Test, or X^2 as appropriate. ANOVA test with Bonferroni correction was used for multiple comparisons (significance level set for $P < 0.05$). Multiple linear regression analysis was used to analyze the relationship between fatty liver, C-IMT and 10-year FRS. Variables in FRS or FLI were not included in multivariate models.

Longitudinal study. The evolution of clinical and biological variables between baseline and follow-up were compared using paired sample T-test for continuous variables and McNemar test for categorical variables. Patients were divided according to the transition between steatosis categories during follow-up, i.e. patients without steatosis at baseline and follow-up, patients with steatosis at baseline and follow-up, steatosis occurrence (FLI < 60 at baseline and ≥ 60 at follow-up) and steatosis regression (FLI ≥ 60 at baseline and < 60 at follow-up). To determine the impact of steatosis on the occurrence of carotid plaques we used Kaplan-Meyer and Cox multivariate analysis models.

All statistical tests were two-sided and significance level was set at $P < 0.05$. Statistical analyses were performed using IBM SPPS 21 MacOS statistical software (Chicago IL).

RESULTS

Relationship between steatosis, carotid atherosclerosis and 10-year FRS (Transversal Study)

5671 patients had available carotid ultrasound and met the inclusion and exclusion criteria (transversal cohort). Patient characteristics are shown in **Table 1**. 50% of patients had more than 2 cardiovascular risk factors and 37% had the metabolic syndrome. Half of the entire cohort had a family history of cardiovascular disease. Mean alcohol consumption was low, only 5% of patients consumed more than 30 g/day. The prevalence of carotid plaques was 39% and the mean FRS was 10±8.

A third of patients (n = 1871, 33%) had a FLI≥60 and were considered to have steatosis. Patients with steatosis were older, had higher BMI and waist circumference, higher prevalence of type 2 diabetes and high blood pressure, higher aminotransferase levels and hsCRP than those without NAFLD. They also had had higher C-IMT and 10-year FRS (**Table 1**). Both C-IMT and FRS progressively increased across FLI quartiles (0.58 ± 0.12 mm, 0.61 ± 0.14, 0.63 mm ± 0.14, 0.64 ± 0.14 mm, $p < 0.001$ and 5 ± 5%, 9 ± 6%, 12 ± 8%, 15 ± 9%, p, 0.001, respectively, **Figure 1A and 1B**).

As type 2 diabetes, obesity and dyslipidemia are independently related to cardiovascular events, we further examined the impact of steatosis on C-IMT and FRS according to the presence of classical cardiovascular risk factors. While diabetics and non-diabetics had different C-IMT values (0.63 ± 0.15 mm vs. 0.61 ± 0.13 mm, respectively, $p<0.001$) this difference disappeared when taking steatosis into consideration. Among patients with type 2 diabetes or dyslipidemia, those with steatosis had significantly higher C-IMT than those without steatosis (0.64 ± 0.14 vs. 0.61 ± 0.14 mm, respectively < 0.001). In contrast, in patients with steatosis, the diabetes and dyslipidemia status were not associated with increased C-IMT (**Table 2**). Results were similar for FRS thus confirming that both the presence of pre-atherosclerotic lesions and the cardiovascular risk score were impacted to a larger extent by the presence of NAFLD than by the presence of diabetes or dyslipidemia (**Table 2**).

Since ALT is a biochemical surrogate for hepatic necroinflammation and since some studies have shown that patients with NASH might be at higher cardiovascular risk than those with steatosis

alone, we studied whether the impact of NAFLD was independent from that of ALT. In this cohort, 45% of patients had normal-low ALT, 38% had normal-high ALT and 17% had high ALT. C-IMT values did not differ significantly according to ALT categories. Patients with steatosis had higher C-IMT levels than patients without steatosis and this was true across all ALT categories (**Figure 2A**). Patients with steatosis had also higher FRS than patients without steatosis, regardless of the ALT category (**Figure 2B**).

In univariate analysis, age, sex, BMI, type 2 diabetes, high blood pressure and steatosis assessed by FLI were independent predictors of C-IMT, **Supplementary Table 1**. In multivariate analysis, FLI predicted C-IMT independent of the metabolic comorbidities, cardiovascular risk factors and markers of low grade inflammation, (beta = 0.046, p = 0.002), **Table 3**. However, when FLI was replaced by its individual components, none of them, except for waist circumference, were independently associated with C-IMT (**Table 3**).

FLI was also associated with 10-year Framingham Score independent of C-IMT (beta=0.432, $p < 0.001$, R^2=0.257); similar results were obtained in diabetic patients only (N = 962, beta=0.269, $p < 0.001$).

Impact of fatty liver on the progression of carotid atherosclerosis (longitudinal study)

Repeat C-IMT measurements were available in 1872 patients (longitudinal cohort, mean time interval 8 ± 4 years). Patients with available repeat C-IMT were younger, had lower BMI, waist circumference transaminases level and lower prevalence of steatosis but similar C-IMT and prevalence of carotid plaques. (**Supplementary Table 2**). In patients with control carotid ultrasound, changes in clinical and biological parameters during follow-up are shown in **Supplementary Table 3**. There was a significant tendency towards weight gain, increase in abdominal girth and a higher proportion of type 2 diabetes. At the same time these patients were more massively treated with lipid lowering agents (73% of them vs. 47% at beginning of follow-up) which resulted in a better control of lipid disorders, particularly, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. The prevalence of

steatosis increased significantly during follow-up (24% at baseline vs. 30%, p<0.001). Steatosis occurred in 12% of patients and regressed in 6% at a crude rate of 0.75% per year. In parallel with the increasing proportion of steatosis, there was a degradation in liver enzymes as shown by increasing values of aminotransferases and GGT during follow-up. Interestingly, most of this increase was related to the occurrence of steatosis, since changes in liver enzymes were higher in patients that developed steatosis during follow-up. The prevalence of carotid plaques also increased during follow-up (39% vs. 57%, p<0.001), with 23% of patients developing carotid plaques vs. 5% no longer having them at the end of follow-up (**Supplementary Table 3**).

Overall, C-IMT increased from 0.61 ± 0.14 mm to 0.64 ± 0.13 mm, p<0.001. Patients with steatosis had higher C-IMT values at baseline than those without steatosis (0.60 ± 0.13 vs. 0.64 ± 0.14 mm, p<0.05). Patients with steatosis occurrence during follow-up had similar C-IMT at baseline as those that stayed free of steatosis (0.60 ± 0.13 vs. 0.61 ± 0.13 mm, p=1); however, at follow-up, their C-IMT was significantly higher 0.66 ± 0.14 vs.0.63 ± 0.13, p = 0.020.

Steatosis at baseline predicted incident carotid plaques during follow-up, as shown in **Figure 3** by Kaplan-Meier analysis. In a multivariate Cox regression model, steatosis at baseline predicted incident carotid plaques independent of age, sex, type-2 diabetes, high blood pressure, tobacco use, hsCRP, and baseline C-IMT (**Table 4**). Even after adjustment for lipid, cardiovascular and diabetes therapies, steatosis at baseline was still an independent predictor of the occurrence of carotid plaques during follow-up (OR = 1.56, 95% CI 1.05 – 2.31, p = 0.027).

Discussion

Patients with NAFLD die primarily of liver disease, and the extent to which the liver disease rather than associated comorbidities is responsible for excess cardiovascular death is still under debate[16]. Most studies have detailed the relationship between NAFLD, associated cardiometabolic risk factors and cardiovascular events. However, clinical events are by definition a late step in the atherogenic process, which makes it difficult to ascertain the contribution of NAFLD per se. If NAFLD were to play an independent role in the development of atherosclerosis, then it should promote the occurrence and progression of early, pre-atherosclerotic lesions. Such long-term, observational data are, however, limited.

In this study we demonstrated that steatosis, as assessed by FLI, a well-validated biomarker panel, is associated with C-IMT, a pre-atherosclerotic lesion that predicts cardiovascular events. C-IMT increased proportionally with FLI, and this association was independent of traditional cardiometabolic risk factors. Steatosis identified patients with higher C-IMT better than type 2 diabetes or dyslipidemia. Importantly, patients that remained free of steatosis had lower baseline and follow-up C-IMTs, while those that had an occurrence of steatosis had the highest follow-up C-IMT values. Steatosis predicted the occurrence of carotid plaques during follow-up and was associated with higher cardiovascular risk at all times, both baseline and follow up, as assessed by the FRS. Collectively, these data support a critical role for steatosis in the progression of atherosclerotic disease, a role that is largely independent of traditional risk factors. Since these data are derived from an at-risk primary prevention cohort rather than a highly selected population that had already experienced cardiovascular events, the conclusions of this study apply to the large majority of patients with NAFLD from the general population. In the current series, the mean C-IMT was rather low (0.62 ± 0.13 mm, range from 0.28 to 1.6 mm). However, even lower levels of C-IMT have additive predictive value for cardiovascular events, especially in asymptomatic individuals at intermediate cardiovascular risk [17-19] .

Several other studies have documented the association between NAFLD and pre-atherosclerotic lesions or carotid plaques. In a landmark paper, Marchesini et al. showed that patients with steatosis and steatohepatitis have altered flow-mediated vasodilation of the brachial artery and a higher cardiovascular risk as measured by the FRS [20]. Several reports have now shown that NAFLD increases the risk of coronary artery calcium scores, a sensitive indicator of early atherosclerosis, independent of features of the metabolic syndrome or conventional cardiovascular risk factors [8, 21]. An association between NAFLD and arterial stiffness has been documented in several studies [22-24], although in adolescents NAFLD was associated with increased arterial stiffness only in individuals with high-risk metabolic profiles [25]. NAFLD has also been associated with carotid plaques compared to an age-, sex- [26] and sometimes BMI- matched control groups [27]. Similar to the present report, a previous study has shown that NAFLD is associated with increased C-IMT vs. age-, sex- and BMI-matched controls [28] and that this correlation is independent of potential confounders. This case-control study was much smaller than the current series, but NAFLD was diagnosed histologically and the authors were able to document significant associations between C-IMT and the degree of the main histological lesions of NAFLD, mainly steatosis, liver inflammation and fibrosis[28]. C-IMT is a non-invasive ultrasound marker of early atherosclerosis [29] that predicts cardiovascular events in the general population, independent of all major risk factors [10]. C-IMT is also an accepted surrogate for cardiovascular events that is strongly predictive of cardiovascular morbidity and mortality events, and hence recommended as a surrogate end-point for outcome cardiovascular trials [30]. Our study confirms the independent relationship between steatosis and C-IMT and provides important longitudinal data supporting this association in a very large population seen in a primary prevention center. The association between NAFLD and early atherosclerosis is corroborated in the same population by the prediction of occurrence of carotid plaques and by higher cardiovascular risk scores. One other report confirmed in a large transversal study that FLI predicted carotid plaques independent of age and smoking [31]. However that study involved a healthy population devoid of any metabolic comorbidities; it thus did not address the question of whether steatosis by itself,

independent of metabolic comorbidities, increases the risk of early atherosclerosis in a typical population of individuals at risk for NAFLD with one or several features of the metabolic syndrome.

Numerous studies have documented the predictive value of altered liver enzymes over the development of cardiovascular complications, both for GGT and aminotransferases [32-35]. Most of these studies, however, dealt with clinical events and not pre-atherosclerotic lesions. Siddiqui et al. showed that serum levels of aminotransferase were associated with increased atherogenesis, however their analysis did not take steatosis into account [36]. Interestingly, in our series, when controlling for steatosis, neither aminotransferases nor GGT were associated with C-IMT or carotid plaques or with the occurrence of carotid plaques in the follow-up study.

This study contributes to the growing body of evidence that NAFLD can predate the phenotypical complications associated with insulin resistance and the clinical features of the metabolic syndrome [37]. NAFLD predicts cardiovascular disease but also type 2 diabetes and arterial hypertension [38-40]. The pathogenesis of these clinical observations has been partly elucidated. The fatty and inflamed liver results in hyperglycemia [41, 42] and overproduces triglyceride-rich VLDL particles, which in turn leads to low HDL-cholesterol and increased small-density LDL particles [43], a pro-atherogenic profile. It also overproduces coagulation factors, including fibrinogen, which increases the risk of thromboembolic events [44]. Regardless of the mechanisms involved, the clinical implications are of first-hand importance, since patients at cardiovascular risk carrying one or several features of the metabolic syndrome have further increased risk if they have steatosis. Also patients with steatosis but only limited overweight and no type 2 diabetes or arterial hypertension are at higher risk of developing these complications than those without steatosis, which makes NAFLD a precursor of the metabolic syndrome. It follows that the diagnosis of steatosis is critical, and therefore a thorough cardiovascular and metabolic work-up and a strict monitoring of cardiovascular disease or of metabolic complications are needed in the clinical management of NAFLD patients. Whether the reduction of steatosis or the reversal of NAFLD will help improve the cardiometabolic risk is unknown, but it will be of critical importance as NASH therapies become available [45]. It is worth

noting that in our longitudinal study, NAFLD predicted incident carotid plaques, even after adjustment for lipid-lowering or diabetes therapy. In NAFLD patients, C-IMT progressed despite a massive increase in the proportion of patients on statins during follow-up. This would suggest that correcting for the metabolic conditions might not be enough to minimize the risk of carotid atherosclerosis as long as liver steatosis is still present.

This study has several limitations. It is a retrospective study but the data was systematically collected in a well-defined cohort, and the risk of selection bias appears minimal. Not all patients had a follow-up carotid ultrasound. Steatosis was assessed by a surrogate biochemical marker and not histologically or by magnetic resonance spectroscopy. In large cohorts however, an invasive procedure or a complex, costly imaging method would not be feasible. Instead, FLI is a well-accepted surrogate of steatosis and has been validated in the general population, with an estimated accuracy of 84% [13]. When compared to liver biopsy, FLI discriminated between the presence or absence of > 5% steatosis with good accuracy [46]. In addition many studies have shown that FLI also predicts overall and hepatic mortality, accelerated atherosclerosis, cardiovascular risk, insulin resistance and incident diabetes [47-49]. Interestingly, in the current study, FLI performed better than its individual components for both the association and the prediction of early atherosclerosis. This further strengthens its validity as a surrogate for steatosis and not merely a biochemical panel that would simply represent the sum of its parts. Unfortunately, because histological data were not available, this study cannot provide evidence as to whether steatohepatitis, which combines steatosis, hepatic inflammation and liver cell injury, has a stronger association with early atherosclerosis than steatosis alone.

In conclusion, in this large cohort of patients at cardiovascular risk from a primary prevention program, steatosis was associated with lesions of early atherosclerosis, independent of traditional cardiovascular risk factors, both in transversal and longitudinal studies. This confirms the deleterious role of hepatic fat accumulation in the occurrence and worsening of features of the metabolic syndrome, which places patients at high cardiometabolic risk. From a metabolic point of view, steatosis is not an innocent bystander but rather a driving force, and hence an exhaustive work-up of

the liver and cardiovascular and metabolic complications, strict monitoring and possibly reinforced therapies should be implemented.

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Legend for Figures:

Figure 1. Distribution of C-IMT (A) and of Framingham risk score (B) according to quartiles of FLI values.

Figure 2. Distribution of C-IMT (A) and Framingham Score (B) according to the presence of steatosis and ALT categories. Low-normal ALT: <19 IU/l in women and <30 IU/l in men; high-normal ALT: between 19 IU/l and 40 IU/l in women and 30 IU/l and 40 IU/l in men; High ALT: >40 IU/l both in men and in women

Figure 3. Impact of baseline steatosis on the occurrence of carotid plaques during follow-up.

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Table 1. Patient characteristics according to the presence of steatosis.

	All	With steatosis	Without steatosis	P
	N = 5671	N = 1871	N = 3800	
Age, years (mean±sd)	52 ± 11	53 ± 10	52 ± 11	< 0.001
Sex, male, %	53	65	46	< 0.001
BMI, kg/m^2 (mean±sd)	26.1 ± 4.7	30.5 ± 4.6	24 ± 3	< 0.001
BMI ≥ 25 kg/m^2, %	55	93	36	< 0.001
Waist circumference, cm, (mean±sd)	90 ± 14	104 ± 10	84 ± 10	< 0.001
Type 2 diabetes, %	17	23	14	< 0.001
High blood pressure, %	41	49	37	< 0.001
Metabolic syndrome (%)	37	69%	21	< 0.001
Family history of cardiovascular disease (%)	49	43	52	< 0.001
Alcohol, g/day, (mean±sd)	8 ± 11	9 ± 13	7 ± 11	< 0.001
Smoking status (former or active), %	46	51	43	< 0.001
AST, IU/l (mean ± sd)	29 ± 14	34 ± 18	26 ± 11	< 0.001
ALT, IU/l (mean±sd)	30 ± 20	40 ± 27	25 ± 13	< 0.001
GGT, IU/l (mean±sd)	40 ± 53	65 ± 80	26 ± 23	< 0.001
Ferritin, ng/ml (mean±sd)	170 ± 182	216 ± 216	145 ± 155	< 0.001
Fasting glucose, mmol/l (mean±sd)	5.3 ± 1.1	5.7 ± 1.4	5 ± 0.9	< 0.001
Total cholesterol (mmol/l)	6.17 ± 1.44	6.16 ± 1.58	6.17 ± 1.37	0.703
Triglyceride, mmol/l (mean±sd)	1,67 ± 1.65	2.6 ± 2.4	1.19 ± 0.7	< 0.001
LDL, mmol/l (mean±sd)	4.06 ± 5.6	3.9 ± 7	4.1 ± 4.7	0.334
HDL, mmol/l (mean±sd)	1.44 ± 0.5	1.2 ± 0.38	1.5 ± 0.5	< 0.001
Lipid lowering medication (%)	43	44	43	0.3
hsCRP, mg/l (mean±sd)	2.4 ± 4.3	3.4 ± 4	1.9 ± 4.3	< 0.001
C-IMT, mm (mean±sd)	0.62 ± 0.13	0.64 ± 0.14	0.61 ± 0.13	< 0.001
Carotid plaques (%)	39	44	37	< 0.001
Framingham Score (mean±sd)	10 ± 8	14 ± 9	8 ± 7	< 0.001
FLI	43 ± 31	81 ± 12	25 ± 17	< 0.001

Table 2. Impact of steatosis on C-IMT values and Framingham Score according to the presence of type 2 diabetes and/or dyslipidemia.

C-IMT

	Type 2 diabetes or dyslipidemia		
	Present	Absent	P*
No NAFLD	0.61 ± 0.14	0.60 ± 0.13	1
NAFLD	0.64 ± 0.14	0.63 ± 0.13	1
P**	< 0.001	< 0.001	< 0.001***

FRAMINGHAM SCORE

	Type 2 diabetes or dyslipidemia		
	Present	Absent	P*
No NAFLD	9 ± 7	8 ± 6	< 0.001
NAFLD	15 ± 9	13 ± 8	< 0.001
P**	< 0.001	< 0.001	< 0.001***

* P for rows; ** P for columns; *** P for trend

Table 3. Independent predictors of C-IMT.

	C-IMT (N = 5671)			
	Model 1		Model 2*	
Variable	Beta	P	Beta	P
Age	0.411	< 0.001	0.402	< 0.001
Sex	0.126	< 0.001	0.107	< 0.001
Tobacco	0.007	0.61	0.011	0.44
High Blood pressure	0.063	< 0.001	0.061	< 0.001
Type 2 diabetes	0.012	0.39	0.008	0.56
hsCRP	0.035	0.012	0.023	0.11
NAFLD (FLI ≥ 60)	0.046	0.002	-	-
BMI			0.018	0.53
Waist circumference			0.080	0.009
Triglycerides			- 0.004	0.80
GGT			- 0.007	0.64

Both models are based on linear regression analysis.

*In model 2 FLI was replaced with variables included in its calculation formula.

Table 4. Independent predictors of the occurrence of carotid plaques in Cox multivariate models.

	Occurrence of carotid plaques during follow-up			
	Model 1		***Model 2***	
	OR (95% CI)	P	OR (95% CI)	P
Age	1.01 (0.98 – 1.03)	0.35	1.010 (0.989– 1.032)	0.352
Male sex	0.61 (0.43 – 0.88)	0.008	0.65 (0.45 – 0.94)	0.022
Tobacco	1.10 (0.78 – 1.55)	0.57	1.07 (0.75 – 1.51)	0.70
Type 2 diabetes	0.84 (0.54 – 1.33)	0.47	0.88 (0.56 – 1.39)	0.59
Arterial hypertension	1.04 (0.73 – 1.47)	0.81	0.87 (0.57 – 1.34)	0.55
Baseline C-IMT	2.21 (0.67 – 7.25)	0.18	2.42 (0.71 – 7.91)	0.14
hsCRP	0.95 (0.89 – 1.01)	0.14	0.95 (0.89 – 1.01)	0.16
Steatosis at baseline	1.63 (1.10 – 2.41)	0.014	1.56 (1.05 – 2.31)	0.027
Lipid lowering treatment	-	-	0.80 (0.56 – 1.13)	0.20
Type 2 diabetes treatment	-	-	0.72 (0.34 – 1.52)	0.39
Cardiovascular treatment	-	-	1.44 (0.82 – 2.54)	0.20

Figure 1A.

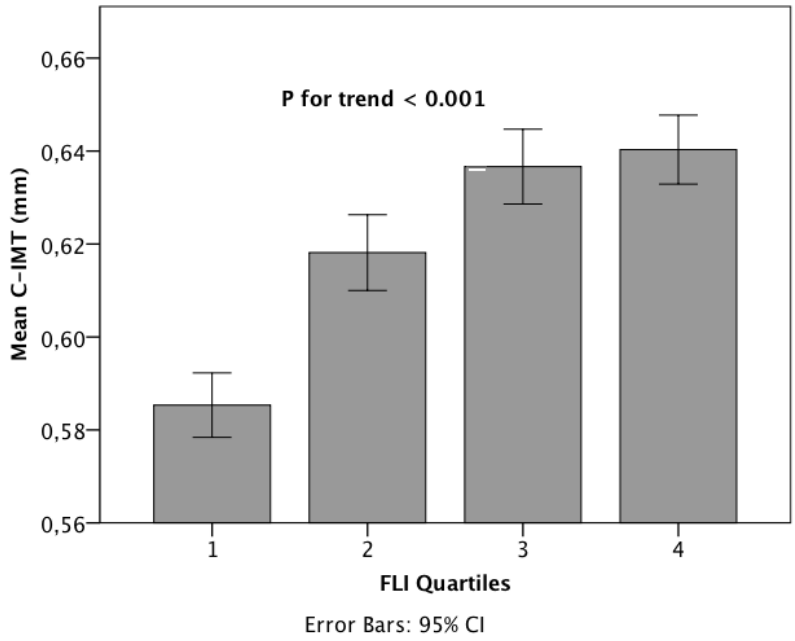


Figure 1B.

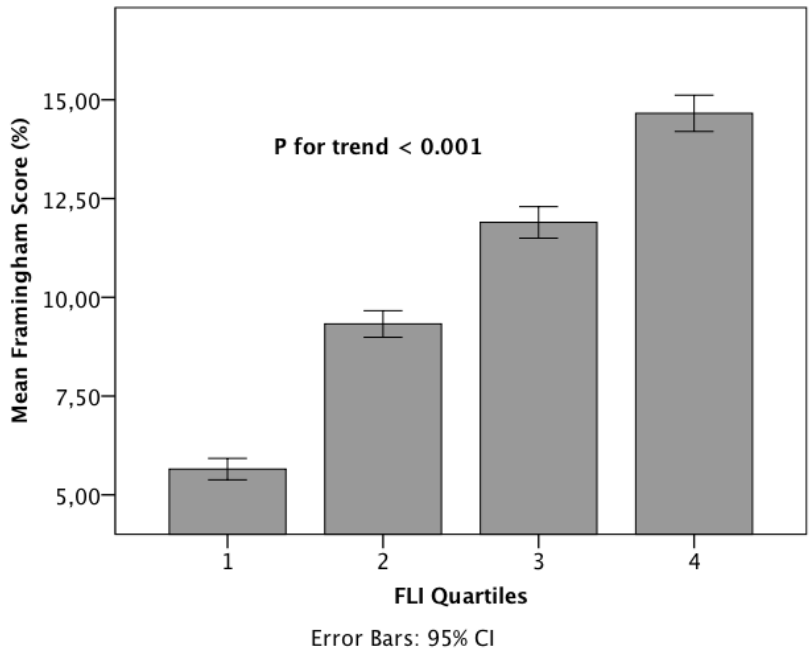


Figure 2A.

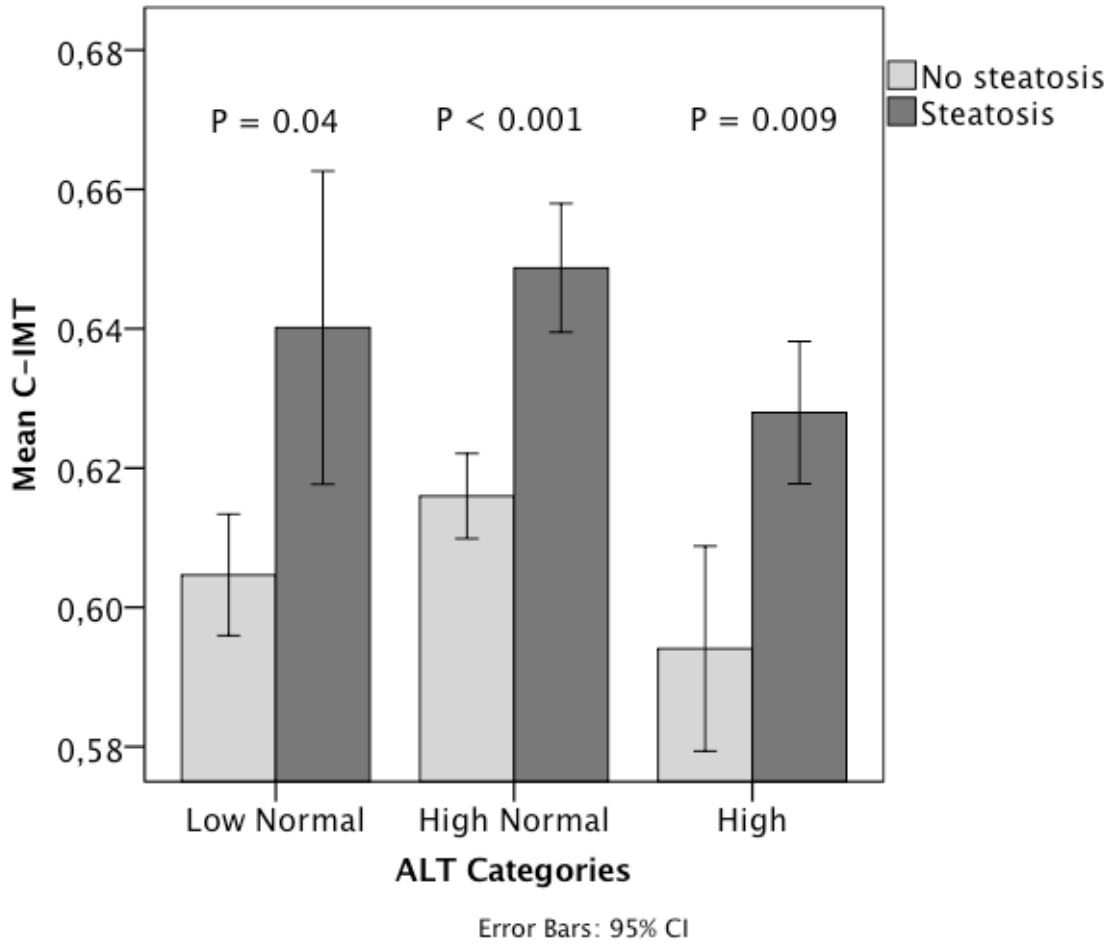

0,68
0,66
0,64
0,62
0,60
0,58
Mean C-IMT
P = 0.04
P < 0.001
P = 0.009
No steatosis
Steatosis
Low Normal
High Normal
High
ALT Categories
Error Bars: 95% CI

Figure 2B.

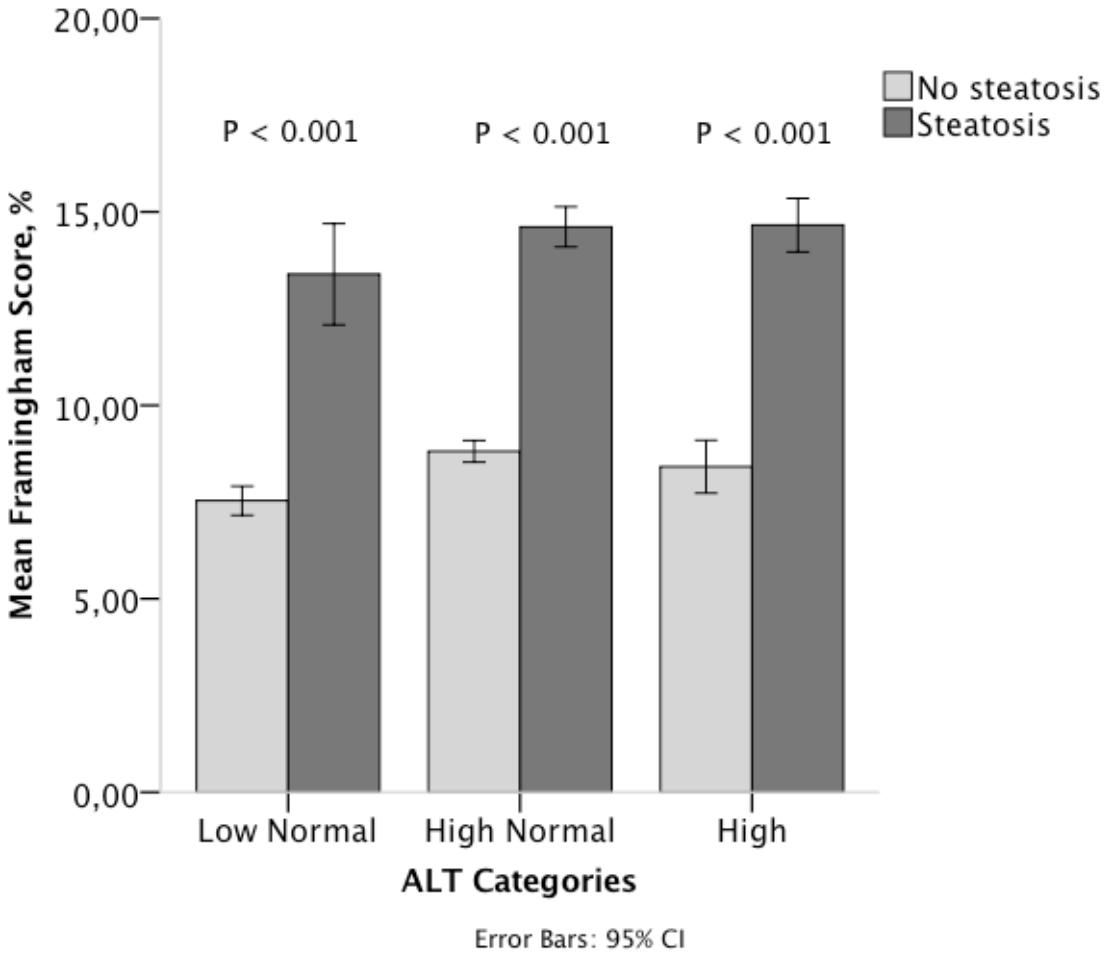
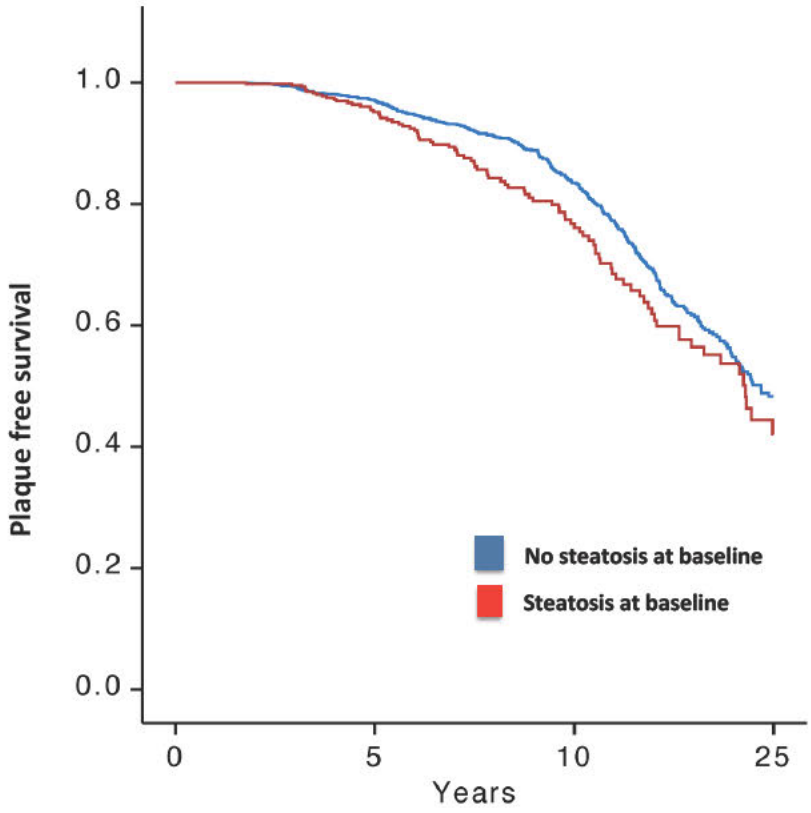

20,00
15,00
10,00
5,00
0,00
Mean Framingham Score, %
No steatosis
Steatosis
P < 0.001
P < 0.001
P < 0.001
Low Normal
High Normal
High
ALT Categories
Error Bars: 95% CI

Figure 3.



No steatosis	1421	1103	512	100
Steatosis	451	301	115	17

Supplementary Tables.

Supplementary Table 1. Variables associated with C-IMT, carotid plaques and Framingham Score by univariate analysis.

	C-IMT		Carotid Plaques		Framingham Score	
	Beta	P	Beta	P	Beta	P
Age	0.393	0.001	0.328	< 0.001	0.397	< 0.001
Sex	0.056	< 0.001	0.029	0.031	0.374	< 0.001
BMI	0.122	< 0.001	0.033	0.013	0.249	< 0.001
Tobacco	0	0.98	0.078	< 0.001	0.363	< 0.001
Type two diabetes	0.051	< 0.001	0.021	0.12	0.113	< 0.001
High blood pressure	0.124	< 0.001	0.095	< 0.001	0.124	< 0.001
Total cholesterol	- 0.034	0.017	0.005	0.728	0.150	< 0.001
CRP US	0.053	0.001	0.034	0.027	0.099	< 0.001
ALT	- 0.002	0.87	0.020	0.13	0.129	< 0.001
AST	0.034	0.015	0.031	0.019	0.109	< 0.001
GGT	0.032	0.024	0.047	< 0.001	0.345	< 0.001
NAFLD (FLI ≥ 60)	0.103	< 0.001	0.062	< 0.001	0.361	< 0.001

Supplementary Table 2. Baseline characteristics of patients with and without repeat C-IMT measurements during follow-up.

	Repeat C-IMT measurements (N = 1872)	**No repeat C-IMT measurements (N = 3799)**	**P**
Age, years (mean±sd)	51 ± 10	53 ± 11	< 0.001
Sex, male, %	57	50	< 0.001
BMI, kg/m^2 (mean±sd)	25.3 ± 4.1	26.6 ± 5	< 0.001
Waist Circumference, cm, (mean±sd)	87 ± 12	92 ± 14	< 0.001
Type two diabetes, %	19	16	0.01
High blood pressure, %	40	42	0.09
Alcohol, g/day, (mean±sd)	9 ± 12	8 ± 11	< 0.001
Smoking status (active), %	48	44	0.009
AST, IU/l (mean ± sd)	26 ± 15	30 ± 14	< 0.001
ALT, IU/l (mean±sd)	28 ± 18	31 ± 21	< 0.001
GGT, IU/l (mean±sd)	35 ± 51	41 ± 54	< 0.001
Ferritin, ng/ml (mean±sd)	208 ± 197	159 ± 175	< 0.001
Fasting Glucose, mmol/l (mean±sd)	5.2 ± 1	5.3 ± 1.1	0.005
Total Cholesterol (mmol/l)	6.45 ± 1.4	6.02 ± 1.45	< 0.001
Triglyceride, mmol/l (mean±sd)	3.6 ± 3.6	4.3 ± 4.3	< 0.001
LDL, mmol/l (mean±sd)	4.3 ± 1.3	3.8 ± 1.2	< 0.001
HDL, mmol/l (mean±sd)	1.4 ± 0.4	3.7 ± 1.2	< 0.001
Lipid lowering medication (%)	47	41	< 0.001
hsCRP, mg/l (mean±sd)	2.4 ± 5.8	2.4 ± 3.7	0.74
C-IMT, mm (mean±sd)	0.61 ± 0.14	0.62 ± 0.13	0.41
Carotid plaques (%)	39	40	0.76
Framingham Score (mean±sd)	11 ± 8	10 ± 8	< 0.001
Fatty Liver Index	37 ± 29	46 ± 31	< 0.001
NAFLD (FLI ≥ 60), %	24	37	< 0.001

Supplementary Table 3. Changes in clinical, metabolic and biological features between baseline and follow-up carotid ultrasound.

	Baseline	Follow-up	P
BMI, kg/m^2 (mean±sd)	25.3 ± 4.1	26 ± 4.1	< 0.001
Waist circumference, cm, (mean±sd)	87 ± 12	93 ± 12	< 0.001
Type 2 diabetes, %	19	29	< 0.001
High blood pressure, %	40	40	0.6
Alcohol, g/day, (mean±sd)	9 ± 12	9 ± 11	0.2
Smoking status (former or active), %	48	47	0.4
AST, IU/l (mean ± sd)	26 ± 15	30 ± 13	< 0.001
ALT, IU/l (mean±sd)	28 ± 18	31 ± 18	< 0.001
GGT, IU/l (mean±sd)	35 ± 51	41 ± 50	< 0.001
Ferritin, ng/ml (mean±sd)	208 ± 198	202 ± 212	0.2
Fasting glucose, mmol/l (mean±sd)	5.2 ± 1	5.3 ± 1.1	< 0.001
Total cholesterol (mmol/l)	6.5 ± 1.3	5.7 ± 1.2	< 0.001
Triglyceride, mmol/l (mean±sd)	3.6 ± 3.6	3.4 ± 2.7	0.001
LDL, mmol/l (mean±sd)	4.3 ± 1.3	3.6 ± 1.1	< 0.001
HDL, mmol/l (mean±sd)	1.45 ± 0.4	1.48 ± 0.5	< 0.001
Lipid lowering medication (%)	47	73	<0.001
hsCRP, mg/l (mean±sd)	2.4 ± 5.8	1.9 ± 4.1	< 0.05
C-IMT, mm (mean±sd)	0.61 ± 0.14	0.64 ± 0.13	< 0.001
Carotid plaques (%)	39	57	< 0.001
Framingham Score (mean±sd)	11 ± 8	9 ± 6	< 0.001
Fatty Liver Index	37 ± 29	44 ± 27	< 0.001
NAFLD (FLI ≥ 60), %	24	30	< 0.001

VII-2. Résumé de l'article 3.

VII-2.1. Méthodes.

Nous avons étudié rétrospectivement une cohorte de 5671 patients avec au moins deux facteurs de risque CV suivis dans une centre de prévention CV primaire. Les patients avec autres causes connues de maladie hépatique, avec une consommation excessive de l'alcool ainsi que les patients avec antécédents d'évènements CV n'ont pas été inclus.

1872 patients ont eu un suivi longitudinal avec au moins deux mesures de l'EIMc et des plaques carotidiennes.

Les données cliniques et biologiques étaient disponibles au moment de l'échographie carotidienne pour tous les patients. La mesure de l'EIMc a été effectuée par deux médecins spécialisés. Le coefficient de variabilité inter-observateur était < 3%. Le Score de Framingham a été calculé pour chaque patient au moment de la mesure de l'EIMc.

La NAFLD a été définie a l'aide d'un marqueur non-invasif, le « Fatty Liver Index » (FLI) qui a été validé dans de nombreuses études[103]:

FLI = ($e^{0.953*\log e(\text{triglycérides}) + 0.139*\text{IMC} + 0.718*\log e(\text{GGT}) + 0.053*\text{tour de taille} - 15.745}$) / ($1 + e^{0.953*\log e(\text{triglycérides}) + 0.139*\text{BMI} + 0.718*\log e(\text{GGT}) + 0.053*\text{tour de taille} - 15.745}$) * 100.

Le diagnostique de NAFLD a été établie si le FLI était ≥ 60.

VII-2.2. Résultats principaux et discussion

Etude transversale.

La prévalence du syndrome métabolique, la NAFLD et les plaques carotidiennes dans la cohorte transversale de 5671 patients étaient de 37%, 33% et 39%.

Nous avons confirmé l'augmentation progressive de l'EIMc et de Score de Framingham avec le nombre de facteurs de risque CV.

L'EIMc et le Score de Framingham étaient plus élevés chez les patients avec NAFLD (0.64 ± 0.14 vs. 0.61 ± 0.13, p < 0.001 et 14 ± 9 vs. 8± 7, p < 0.001) et ont augmenté progressivement avec les quartiles de FLI (0.58 ± 0.12 mm, 0.61 ± 0.14, 0.63 mm ± 0.14, 0.64 ± 0.14 mm, p < 0.001 and 5 ± 5%, 9 ± 7%, 12 ± 8%, 15 ± 9%, p, 0.001).

Ces résultats confirment des études antérieures évaluant l'association entre l'EIMc et la NAFLD. Cependant, dans certaines études l'association entre la NAFLD et l'EIMc était atténuée après ajustement pour le syndrome métabolique. Dans l'étude de Kim et al., l'association entre la NAFLD et l'EIMc était plus faible chez les patients sans syndrome métabolique[285].

Dans cette étude, nous avons démontré que la NAFLD était un facteur de risque indépendant pour l'athérosclérose carotidienne et le Score de Framingham après ajustement pour les facteurs de risque CV classiques ou le syndrome métabolique.

Pour renforcer ces résultats, nous avons effectué des analyses de sous-groupe en fonction de la présence ou l'absence du syndrome métabolique. L'EIMc et le Score de Framingham étaient significativement plus élevés chez les patients sans syndrome métabolique avec NAFLD que chez les patients sans syndrome métabolique et sans NAFLD (0.62 ± 0.13 vs. 0.59 ± 0.12, p < 0.0010 et 8 ± 6 vs. 12± 8, p < 0.001).

Dans l'analyse multivariée, après ajustement pour l'âge, le sexe, la consommation de tabac, le diabète type 2, l'hypertension artérielle et la CRPus, FLI était un facteur prédictif indépendant de l'EIMc (beta = 0.046, p = 0.002).

Ces résultats renforcent l'hypothèse que la NAFLD est un facteur de risque CV indépendant de syndrome métabolique. Dans une revue systématique récente qui a analysé les données de 3497 patients provenant des 7 études évaluant l'association entre la NAFLD et l'EIMc, les patients avec NAFLD avait une augmentation de 13% de l'EIMc en comparaison avec les patients sans NAFLD[299]. Le diagnostic de NAFLD a été prouvé histologiquement dans 3 études et établi par échographie dans 4 autres études. La sévérité des lésions histologiques semble être significativement corrélée avec l'EIMc[281].

Ces résultats renforcent les données épidémiologiques qui suggèrent que, contrairement aux patients avec stéatose isolée, les sujets avec NASH ont une augmentation significative du risque CV[164].

Plus récemment une autre étude portant sur 1000 sujets a confirmé l'association entre la NAFLD et l'augmentation de l'EIMc. Similaire avec notre étude, le diagnostic de la NAFLD a été établi par un FLI > 60[335].

Nos résultats confirment la valeur prédictive de l'EIMc pour le risque CV estimé par le Score de Framingham (beta = 0.272, $p < 0.001$, $R^2 = 8\%$). La valeur moyenne de l'EIMc était plutôt basse dans notre cohorte (0.62 ± 0.13 mm). Cependant, des études antérieures ont montré que même des valeurs bases de l'EIMc ont une valeur prédictive positive pour des évènements CV futurs[338-340]. Nous avons démontré que la NAFLD avait une valeur prédictive pour le Score de Framingham indépendante de l'EIMc (beta = 0.345, $p < 0.001$).

Afin d'explorer l'association entre la NAFLD et le risque CV nous avons réalisé des analyses de sous-groupe chez les patients diabétiques ou obeses.

Dans notre cohorte 962 patients étaient diabétiques de type 2. L'EIMc et le Score de Framingham étaient significativement plus élevés chez les patients diabétiques avec NAFLD que chez ceux sans NAFLD (0.64 ± .14 mm vs. 0.61 ± 0.14 mm, $p < 0.001$ et 15 ± 9 vs. 9 ± 7, $p < 0.001$).

La relation entre la stéatose et l'EIMc chez les patients diabétiques reste sujet de controverse. Dans une large cohorte des patients avec diabète type 2, Targher et al., avait montré que les patients avec NAFLD avait une prévalence plus élevée des maladies coronaires, cérébrovasculaires ou vasculaire périphériques, indépendamment du syndrome métabolique, du contrôle et du traitement du diabète ou des autres facteurs de risque CV classiques (âge, sexe, tabac)[251]. Des résultats similaires ont été obtenus chez les patients avec diabète de type 1[341]. Les mêmes auteurs confirment une association causale indépendante entre la sévérité de la stéatose histologique et l'EIMc chez les patients diabétiques[281]. En revanche, deux autres études n'ont pas trouvé une relation de causalité entre la stéatose (diagnostiquée par CT ou spectro IRM) et l'EIMc. Ces dernières études suggèrent que chez les patients diabétiques, la stéatose est plus un épiphénomène qu'un médiateur direct de l'atherosclerose[286, 300].

Comme dans d'autres études[342, 343] nous avons trouvé une corrélation significative entre l'EIMc, le Score de Framingham et l'obésité. La NAFLD associée à l'obésité augmente le risque CV (le Score de Framingham était 14 ± 8 chez les patients obeses avec NAFLD et 10 ± 7 chez les patients obeses sans NAFLD, $p < 0.001$). Cet effet est probablement dû à une production plus importante de cytokines pro–inflammatoires par le tissu adipeux enflammé chez les patients avec NAFLD. Les cytokines pro-inflammatoires sont responsables non seulement de la progression de la maladie

hépatique mais aussi du développement des complications extra hépatiques et particulièrement de l'augmentation du risque CV[344, 345].

Nous n'avons pas trouvé une corrélation significative entre le profil lipidique et l'EIMc. Cependant ces résultats doivent être interprétés avec précaution. Une proportion significative (43%) de patients dans notre cohorte était sous traitement par statines en prévention CV primaire. Des études antérieures ont démontré que le traitement par statines diminue significativement la progression des plaques d'athérosclérose. Ces résultats sont vraisemblablement dus aux effets pléiotropes des statines qui diminuent l'inflammation dans la plaque d'athérosclérose[346, 347].

Etude longitudinale.

Nous avons démontré dans ce travail, la valeur pronostique de la NAFLD sur la progression de l'athérosclérose et la survenue des plaques carotidiennes.

Sur une période de 8 ans (moyenne 8 ± 4 ans), les patients qui avaient une NAFLD en début de suivi, avaient une EIMc et un Score de Framingham significativement plus élevés en fin de suivi (0.64 ± 0.14 mm vs. 0.67 ± 0.14 mm, p = 0.002).

L'étude montre également une augmentation significative de la prévalence de la NAFLD dans l'intervalle de suivi, parallèlement à l'augmentation de l'index de masse corporelle et du tour de taille (survenue de NAFLD chez 12% des patients).

Globalement la prévalence des plaques carotidiennes a augmenté pendant la période de suivi de 39% à 57% ($p < 0.001$). Remarquablement, la prévalence des plaques carotidiennes a augmenté malgré une diminution des taux de cholestérol (73% des patients avaient un traitement hypolipémiant en fin de suivi). Chez les patients qui

avaient une NAFLD en début du suivi, la prévalence des plaques a augmenté de 36% a 64% ($p < 0.001$).

Ces résultats suggèrent qu'en présence de la NAFLD, le contrôle des facteurs de risque métaboliques sans régression de la stéatose n'est probablement pas suffisant pour prévenir la progression de l'athérosclérose. Cette hypothèse est renforcée par l'observation que la NAFLD a régressée uniquement chez 6% des patients, malgré une prise en charge spécifique du syndrome métabolique.

En analyse univariée, la NAFLD a eu une valeur prédictive significative pour le développement des plaques pendant la période de suivi. Apres ajustement pour l'âge, le sexe, le diabète de type 2 et l'hypertension artérielle, la NAFLD était un facteur prédictif pour la survenue des plaques carotidiennes (OR = 1.29, 95% CI 1.02 – 1.63, p = 0.032).

L'hypothèse que la NAFLD est un facteur de risque indépendant de l'athérosclérose a été renforcée par l'augmentation significative de l'EIMc chez les patients qui ont développé une NAFLD dans l'intervalle de suivi. Parmi ces patients, 24% ont développé des plaques carotidiennes.

Les principales limites de notre étude sont le design rétrospectif et l'utilisation d'un marqueur non-invasif (FLI) pour le diagnostic de la NAFLD. Toutefois, le FLI a été validé dans la population générale pour le diagnostic de la stéatose[103] et des études ultérieures ont démontre une corrélation significative entre le FLI, l'athérosclérose carotidienne[334, 335] et la mortalité globale et spécifique[271].

En conclusion, nos résultats suggèrent que la NAFLD intervient comme facteur indépendant dans le processus d'athérosclérose et contribue à la progression de l'athérosclérose carotidienne et à l'augmentation du risque CV au delà de l'association fréquente avec le syndrome métabolique et de la présence des facteurs de risque CV classiques (tel que l'âge, le sexe, le tabagisme).

Chapitre VIII. Conclusions générales, impact clinique et futures directions.

Malgré beaucoup d'avancés dans les dernières années, l'histoire naturelle de la NAFLD est encore mal connue, particulièrement les facteurs expliquant les variations individuelles qui font que certains patients progressent et développent des complications hépatiques ou extra-hépatiques alors que d'autres ne progressent pas et suivent une évolution bénigne.

Il a été précédemment démontré que l'accumulation des triglycérides dans le foie est un phénomène bénin, physiologique, qui est plus un marqueur qu'une cause de la résistance a l'insuline. En revanche, les acides gras libres représentent les formes « agressives » des lipides, responsables de la lipotoxicité, la production des radicaux libres d'oxygène et des cytokines pro-inflammatoires. L'inflammation du tissu adipeux, le microbiome intestinal et les facteurs génétiques jouent un rôle majeur dans l'évolution et progression de l'inflammation hépatique.

Dans ce travail clinique nous avons démontré que l'inflammation hépatique et l'état inflammatoire chronique associé à la NAFLD ont un rôle majeur non seulement dans la progression de la maladie vers ses formes plus avancées de NASH avec fibrose, mais aussi dans la carcinogenèse hépatique et le développement des complications extra-hépatiques, particulièrement cardiovasculaires (**Figure 14**)

INFLAMMATION HEPATIQUE ET SYSTEMIQUE

RESULTATS	IMPACT CLINIQUE	DIRECTIONS FUTURES
Les patients avec NAFL et minimes lésions d'inflammation lobulaire/ portale ou FPS minime sont à risque pour la progression de la maladie	✓ Ces malades doivent bénéficier d'un suivie rapproché et de mesures thérapeutiques spécifiques. ✓ La résolution de l'inflammation hépatique et de la NASH est une cible thérapeutique importante	✓ Eudes prospectives à long-terme avec: ✓ analyse des facteurs génétiques et du microbome intestinal ✓ analyse de la morbidité et mortalité globale et spécifique
Les facteurs de risque métabolique sont fréquents et augmentent le risque de CHC chez les patients avec MAF au stade de cirrhose	✓ Identifier les patients à risque élevé de CHC et optimiser les stratégies de surveillance ✓ Le contrôle des facteurs de risque métabolique pourrait diminuer le risque de CHC	✓ Etudes prospectives couvrant tous le spectre de la MAF et prenant en compte les facteurs génétiques et du microbiome intestinal ✓ Etudes expérimentales afin de mieux comprendre les mécanismes moléculaires de l'interaction entre l'alcool et les facteurs de risque métabolique
La NAFLD intervient dans la progression de l'athérosclérose carotidienne et augmente le risque CV indépendamment du syndrome métabolique ou des facteurs de risque CV classiques	✓ Les patients avec NAFLD devraient bénéficier d'une évaluation initiale du risque CV ✓ La régression de la NASH pourrait résulter dans une diminution du risque CV	✓ Etudes prospectives chez des patients avec NAFLD prouvée histologique ✓ Analyse des facteurs génétiques, du microbiome intestinal, des marqueurs d'insulinorésistance et d'inflammation systémique ✓ Inclusion de la morbidité et mortalité CV comme objectifs secondaires dans les essaies thérapeutiques

1. L'inflammation hépatique et la progression vers la NASH et la fibrose avancée (Article 1)

Dans cette étude explorant l'histoire naturelle de la NAFLD dans une série des patients avec biopsies hépatiques de suivi, nous avons démontré que les patients avec NAFL et minimes lésions d'inflammation lobulaire/portale ou minime fibrose perisinusoidale sont à risque de progression de la maladie, surtout en cas de persistance ou d'aggravation des facteurs de risque métabolique. Nos résultats ont été confirmés par d'autre études[130, 132, 133].

Ces résultats mettent clairement en question le dogme classique qui stipule que ces patients ont un cours bénin de la maladie et ne sont pas à risque de progression.

Nos résultats suggèrent que ces malades, même avec une inflammation minime, doivent bénéficier d'un suivi rapproché et de mesures thérapeutiques spécifiques. La résolution de l'inflammation hépatique et de la NASH est une cible thérapeutique importante, afin de prévenir la progression ultérieure de la fibrose.

Néanmoins notre étude ainsi que des études similaires déjà publiées sont des études rétrospectives réalisées sur un nombre limité des patients.

Dans toutes ces études la définition de la NAFL n'est pas homogène, certaines ayant utilisé le concept de borderline NASH d'autres de NAFL. L'indication pour la biopsie de suivi varie d'une étude à l'autre : persistance des transaminases élevées avec la présence concomitante des facteurs de risque métabolique ; échec d'implémentation des mesures hygieno-diététiques ; inclusion dans des essais thérapeutiques. De plus, bien souvent, les facteurs de risque associés à la progression de la maladie ont été incomplétement analysés. Par exemple, l'ethnicité n'est pas renseignée dans la plupart des études. Il est connu que certains groupes ethniques sont plus à risque que les autres pour développer

une NAFLD, probablement en relation avec un héritage génétique qui est diffèrent entre les ethnies[348, 349]. Les facteurs génétiques, dont certains comme le polymorphisme PNPLA3 ou plus récemment celui du TM6SF2, ont été associés à des formes plus sévères de la maladie mais n'ont pas été analysés dans la plupart des études avec suivi histologique, qui sont souvent plus anciennes que l'identification de ces facteurs génétiques.

D'autres facteurs confondants importants qui peuvent intervenir dans l'histoire naturelle de la NAFLD n'ont pas été renseignés dans les études avec biopsies hépatiques de suivie chez les malades ayant une NAFLD: des informations concernant l'activité physique, le régime alimentaire (contenu en acides gras saturées ou polynesaturées, consommation de fructose, consommation du café), etc. Aussi les données de morbidité et mortalité à long terme n'ont pas été analysés dans aucune de ces études.

Vu les limites des études publiées à ce jour, il est nécessaire de réaliser des études prospectives à long terme. Ces études doivent prendre en compte :

- des questionnaires détaillés sur le régime hygièno-diététique (apport en carbohydrates, fructose, acides gras saturés ou polyinsaturés, consommation de café etc.) et le niveau d'activité physique
- les facteurs génétiques (PNPLA3, TM6SF2)
- l'analyse du microbiote intestinal
- donnés de morbidité globale et spécifique (liée au foie, évènements cardiovasculaires, etc)
- donnes de mortalité globale et spécifique

Cela permettra de mieux analyser et comprendre l'histoire naturelle de la NAFLD et de mieux définir le profil des malades à risque pour la progression de la maladie afin de les identifier plus précocement et pouvoir agir en conséquence.

2. L'inflammation hépatique, les facteurs de risque métabolique et la carcinogenèse hépatique

L'obésité et le diabète sont associés à un état d'inflammation chronique qui favorise la carcinogenèse hépatique par des mécanismes multiples : (1) la résistance à l'insuline augmente la biodisponibilité de l'IGF-1 (insulin growth factor) qui favorise la prolifération cellulaire et inhibe l'apoptose[350] ; (2) le stress oxydatif et la lipotoxicité sont responsables de l'altération de la structure de l'ADN et de l'instabilité chromosomale; (3) la production des cytokines pro-inflammatoires (TNF alpha, IL-6) responsables de l'activation des voies pro-oncogènes (mTOR, JNK, STAT3)[220, 351].

Dans ce travail nous avons démontré que les facteurs de risque métabolique sont prévalents chez les patients avec maladie alcoolique du foie et augmentent significativement le risque de carcinome hépatocellulaire même au stade de cirrhose.
Sur le plan clinique, ces résultats peuvent expliquer les différences de prévalence du carcinome hépatocellulaire dans les différentes régions géographiques en fonction de l'exposition différente aux facteurs de risque métabolique.

D'autre part, l'exposition aux facteurs de risque métabolique permet d'identifier un sousgroupe des patients ayant un risque supplémentaire de carcinome hépatocellulaire quelque soit l'étiologie de la maladie chronique du foie et qui pourrait justifier des procédures renforcés de surveillance et de dépistage.

Finalement, l'obésité et le diabète sont des conditions modifiables et amendables aux interventions thérapeutiques spécifiques. On peut spéculer que le contrôle de l'obésité et du diabète par des mesures générales (hygièno-diététiques, perte de poids) ou spécifiques médicamenteuses pourrait résulter en une diminution de l'incidence du carcinome hépatocellulaire via l'amélioration de l'état inflammatoire chronique

responsable de l'activation des voies pro-oncogènes. Des études expérimentales récentes soutiennent cette hypothèse[352].

Il faut admettre que notre étude ainsi que beaucoup d'autres déjà disponibles ont des limites importantes qui dérivent de leur caractère rétrospectif et du nombre limité des sujets analysés. Une des principales difficultés est de définir les facteurs de risque métabolique chez les patients cirrhotiques dont l'état nutritionnel et le métabolisme glucido-lipidique sont fortement modifiés. Notre étude ainsi que des études antérieurs soulignent et renforcent l'impact clinique résultant de l'interaction entre la consommation d'alcool, l'obésité et le diabète mais les mécanismes moléculaires ne sont pas encore complètement élucidés.

Des directions futures de recherche sont : (1) mieux explorer les mécanismes moléculaires d'interactions entre l'alcool et l'obésité et leur impact sur la carcinogenèse hépatique ; (2) d'analyser le rôle du microbiome intestinal et des facteurs génétiques dans le développement du CHC chez les malades ayant une consommation excessive d'alcool ; (3) d'analyser l'interaction entre l'alcool et les facteurs de risque métabolique pour tout le spectre de la MAF (progression de la fibrose, développement de la cirrhose et ses complications).

Des études récentes ont démontré que la concentration plasmatique d'éthanol est fortement corrélée à l'obésité et la résistance à l'insuline expliquant les taux plasmatiques significativement plus élevés chez les patients avec NAFLD[353]. Le métabolisme d'alcool semble être altéré dans les modèles expérimentaux de NAFLD essentiellement en raison d'un déficit de l'activité de l'alcooldehydrogenase (ADH).

Cette hypothèse a été vérifié par des études expérimentales qui montrent : (1) une diminution de 53% de l'activité de l'ADH chez les souris diabétiques[354]; (2) une diminution de l'expression de CYP2E1 et de mARN chez les souris *ob/ob*[355] ; (3) une

diminution de 35% de l'activité de l'ADH chez les souris *ob/ob* non traités vs. ceux traités par des anticorps anti TNF alpha[353]. Ces mécanismes expliquent probablement l'effet synergique de l'alcool, l'obésité et le diabète sur la sévérité de l'atteinte hépatique. Des études chez l'homme sont nécessaires afin de valider ces hypothèses.

Des facteurs génétiques et le microbiome intestinal ont un rôle important dans la sévérité de l'atteinte hépatique et la carcinogenèse. Le polymorphisme de l 'adiponutrine (PNPLA3) intervient dans la progression de la fibrose et le développement de la cirrhose chez les patients avec la NAFLD[47] et la maladie alcoolique du foie[356] et augment significativement le risque de carcinome hépatocellulaire[231].

La consommation chronique d'éthanol ainsi que la résistance à l'insuline augmente la perméabilité intestinale et favorise la translocation d'endotoxines bactériennes (lypopolyzacharide, LPS) de l'intestin. La conséquence est la production excessive des cytokines pro-inflammatoires, l'altération des fonctions de récepteurs FXR (farnesoid X receptors) et l'activation des récepteurs TLR4 (toll-like receptors 4)[357]. Ces mécanismes sont responsables de l'activation des voies de signalisation moléculaire (damage associated molecular patterns, DAMPs), de la prolifération cellulaire et de l'inhibition de l'apoptose, et favorisent la carcinogenèse[358].

Des études expérimentales sur des modèles animaux sont nécessaires afin de mieux comprendre le rôle dans la carcinogenèse hépatique des interactions entre la consommation d'alcool, les facteurs de risque métabolique, les facteurs génétiques et le microbiome intestinal.

Enfin, notre étude a analysé l'impact des facteurs de risque métabolique chez les patients ayant déjà une cirrhose, donc les résultats ne peuvent pas être généralisés pour tout le spectre de la maladie alcoolique du foie. Des études cliniques futures incluant tout le spectre de la maladie alcoolique du foie doivent être réalisés afin de mieux

analyser l'interaction entre l'alcool et les facteurs de risque métabolique et leur impact sur la progression de la fibrose, le développement de la cirrhose et du carcinome hépatocellulaire et sur la mortalité globale et spécifique.

3. L'inflammation hépatique et l'athérosclérose carotidienne

Dans cette étude nous avons démontré que la NAFLD intervient dans la progression de l'athérosclérose carotidienne et augmente le risque cardiovasculaire au delà de l'association fréquente avec le syndrome métabolique ou de la présence des facteurs de risque CV classiques (tel que l'âge, le sexe, ou le tabagisme).

La NAFLD et la l'athérosclérose carotidienne sont associées à un état d'inflammation chronique (due à la dysfonction du tissu adipeux avec production des cytokines pro-inflammatoires, la résistance à l'insuline, le stress oxydatif, le déséquilibre entre les facteurs pro- et anticoagulants) qui contribue au développement et à la progression des deux maladies. Le foie est la source mais aussi l'organe cible de ces mécanismes expliquant à la fois la progression de lésions hépatiques mais aussi le développement des complications extrahépatiques, particulièrement le diabète type 2 et la maladie cardiovasculaire.

A présent, les deux sociétés savantes, Européenne et Américaine, ne détaillent pas suffisamment le besoin d'évaluer le risque CV ou de dépistage pour des signes précoces d'athérosclérose chez les patients avec NAFLD. Cependant, la maladie CV est la première cause de décès chez les malades avec NAFLD avant la mortalité lié au foie. Pour ces raisons, en plus de l'évaluation des facteurs de risque métaboliques, les patients avec NAFLD devraient bénéficier d'une évaluation du risque CV. Compte-tenu du fait que l'EIMc est un marqueur précoce d'athérosclérose avec une valeur prédictive établie pour le risque de développer des évènements CV futurs, l'échographie carotidienne avec mesure de l'EIMc pourrait être recommandée dans l'évaluation des malades avec

NAFLD. Un des points à définir c'est a quel moment de l'histoire naturelle de la NAFLD, la mesure de l'EIMc apporterait le plus des bénéfices pour l'évaluation du risque CV: chez les patients avec stéatose isolée, chez les patients avec NASH ou chez les patients avec NASH et fibrose avancée (≥ F2). Précédemment notre équipe avait montré que la stéatose sévère ainsi que la fibrose ou la progression de la fibrose étaient prédictives pour la survie à 5 ans sans évènements CV chez les patients avec diabète type 2[98]. Une méta-analyse récente n'a pas trouvé des différences significatives pour la survie sans évènements CV majeurs entre les patients avec stéatose isolée ou la NASH[359]. D'autres études montrent une augmentation du risque de morbidité et mortalité CV chez les patients avec NASH mais pas chez les patients avec stéatose isolée. Ces résultats parfois contradictoires suggèrent que probablement tous les malades avec NAFLD devraient bénéficier d'une évaluation initiale du risque CV quel que soit la sévérité de l'atteinte hépatique. Le rythme ultérieur de suivi pour les complications CV et la détection de l'athérosclérose coronarienne précoce pourrait ensuite être établi en fonction de la présence et de la sévérité initiale d'atteinte CV et de la sévérité histologique de la maladie hépatique. Des études prospectives sont nécessaires pour établir l'impact de la NAFLD et de la sévérité des lésions histologiques (stéatose, NASH, fibrose) sur la vitesse de progression de l'athérosclérose carotidienne afin d'élaborer des recommandations pertinentes de screening CV chez ces malades. Egalement il sera intéressant d'évaluer si la régression des lésions histologiques de la NAFLD (par mesures hygièno-diététiques ou traitement pharmacologique spécifique) pourrait diminuer la vitesse de progression de l'athérosclérose carotidienne et en conséquent le risque de développer des évènements CV ultérieurs. Ceci sera au mieux évalué par des études de suivi à long-terme une fois que des médicaments anti-NASH seront disponibles.

Chapitre IX. Références

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Chapitre X. Articles en annexe

Article 1. From NAFLD in clinical practice to answers from guidelines J Hepatol. 2013 Oct;59(4):859-71

Review

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

From NAFLD in clinical practice to answers from guidelines

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Summary

This review of the literature consists of three sections.

First, papers concerning non alcoholic fatty liver disease (NAFLD) awareness among the general population, general practitioners, and liver and non liver specialists were retrieved and analyzed to highlight the perception of disease, verify knowledge of current recommendations, and identify the main difficulties experienced in clinical practice.

Next, position papers and clinical practice guidelines issued by International and National Hepatological Scientific Societies were identified and critically assessed in order to pinpoint the areas of convergence/difference.

Finally, practical suggestions on NAFLD diagnosis and management in daily practice are provided and the open questions highlighted.

Introduction

Non alcoholic fatty liver disease (NAFLD), the hepatic counterpart of the metabolic syndrome (MS) [1,2], encompasses a disease spectrum spanning steatosis through non alcoholic steatohepatitis (NASH) with/without cirrhosis, and hepatocellular carcinoma (HCC) [3]. The obesity and type 2 diabetes (T2D) pandemic and the improved management of chronic viral hepatitis have resulted in NAFLD becoming a leading cause of chronic liver disease (CLD) [4] and a major health concern owing to hepatic and extrahepatic morbidity/mortality [5 7].

Such a shift in the epidemiology of CLD has left practicing clinicians somewhat puzzled in identifying and treating this NAFLD "epidemic" [8 12]. Moreover, an ever increasing number of practice guidelines on NAFLD diagnosis and management issued by eminent Scientific Societies may probably add to the uncertainties concerning the best conduct to follow in clinical practice.

Our paper aims at (1) highlighting the perception of NAFLD among practicing physicians, (2) providing a critical, comparative analysis of the statements on NAFLD diagnosis and management, issued by clinical practice guidelines and technical reviews of Scientific Societies, (3) offering practical suggestions on the controversial topics and defining the unsettled questions.

Methods

We conducted a PubMed database search (keywords: general practice and/or primary care and/or specialists and/or physicians and/or awareness and/or perception and/or liver steatosis and/or fatty liver and/or NAFLD and/or NASH and/or guidelines and/or recommendations. Limits: December 2012 and English language) aimed at ascertaining: (a) the awareness/perception of the importance of NAFLD NASH among potential patients and practicing physicians [both general practitioners (GPs) and specialists] and (b) guidelines/consensus/recommendations for NAFLD diagnosis and management issued by Medical Societies.

Six studies meeting the inclusion and exclusion criteria investigated current beliefs and practices of NAFLD among the general population, GPs and liver and non liver specialists [8 13]. Moreover, three further studies [14 16] addressing the clinical approach of practicing physicians towards pediatric NAFLD were identified (Table 1).

Five position papers and clinical practice guidelines, issued by the European Association for the Study of the Liver (EASL) [17], Asian Pacific Working Party for NAFLD (APWP NAFLD) [18], Chinese Liver Disease Association (CLDA) [19], Italian Association for the Study of the Liver (IASL) [20] and American Gastroenterological Association (AGA) American Association for the Study of Liver Disease (AASLD) American College of Gastroenterology (ACG) [21], were identified. Three out of five such reports are evidence based [19 21]. A single position paper on diagnosis of

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Abbreviations: NAFLD, non-alcoholic fatty liver disease; MS, metabolic syndrome; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; T2D, type 2 diabetes; CLD, chronic liver disease; GPs, general practitioners; EASL, European Association for the Study of the Liver; APWP, Asian-Pacific Working Party; CLDA, Chinese Liver Disease Association; IASL, Italian Association for the Study of the Liver; AGA, American Gastroenterological Association; AASLD, American Association for the Study of Liver Disease; ACG, American College of Gastroenterology; ESPGHAN, European Society for Pediatric Gastroenterology, Hepatology and Nutrition; LB, liver biopsy; US, ultrasonography; IR, insulin resistance; MRFs, metabolic risk factors; AFLD, alcoholic fatty liver disease; LTs, liver tests; CVD, cardiovascular disease.

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Table 1. Analysis of reports from real-life clinical practice.

Author, yr [Ref.]	Methods	Main findings
Leung CM, *et al.*, 2009 [13]	Telephone survey on NAFLD knowledge among 521 subjects randomly selected from the general population in Hong Kong.	Among those interviewed, 83% had never come across the term "NAFLD." Among those who had heard of NAFLD, 42% had no idea about prevalence, 47% knew nothing about clinical presentation, 78% thought that blood tests could provide definite diagnosis, about 50% mistook associated risk factors and 81% perceived their knowledge of NAFLD as inadequate.
Grattagliano I, *et al.*, 2008 [8]	Online questionnaire and clinical survey about NAFLD knowledge and management before and after attending a teaching workshop among 56 GPs in Italy.	Before/after teaching workshop - Questionnaire (%): 4.7/42.7 indicated NAFLD as the first cause of undefined persistent hypertransaminasemia, 70/<10 underestimated NAFLD prevalence in general population, 36.6/76.2 would screen diabetic subjects, 39.5/100 should make diagnosis after exclusion of all other causes of liver steatosis, 23.2/61.9 should manage NAFLD patients with diet and a new check after 6 months, 2.3/80.9 should ask for LB in over 50 diabetic patients with persistent hypertransaminaseima, 78/91 indicated diet as the first approach. 34.1% should avoid statins. Practice check: improvement in screening of risk patients, searching for NASH and managing NAFLD.
Loguercio C, *et al.*, 2011 [10]	5-yr retrospective analysis from 104 GPs and 6550 patients with CLD in Italy.	Drinking habits registered in only 20.4% of CLD patients. 81.9% of patients with undefined CLD were overweight/obese. In patients with liver steatosis (NAFLD + AFLD): alcohol consumption recorded in 30.2%, BMI recorded in 59.5%, US performed in 37.9% of patients. No record of additional tests including insulin, HOMA index, ferritin, GGT, lipids and HBV- HCV markers.
Kallman JB, *et al.*, 2009 [9]	Survey questionnaire about screening for HBV, HCV and NAFLD among 103 GPs, 59 gastroenterologists and 52 hepatologists in USA.	Compared to specialists, GPs significantly less likely to be aware of official guidelines, to rate NAFLD as a common cause of liver disease, to screen for NAFLD in asymptomatic patients with diabetes but believed more strongly that available treatments for NAFLD are effective. Hepatologists endorsed appropriate screening scenarios more frequently than gastroenterologists and GPs.
Bergqvist CJ, *et al.*, 2012 [11]	Face-to-face questionnaire assessing beliefs and practices regarding NAFLD among 100 non-liver specialists in Australia.	75% underestimated the prevalence of NAFLD in the general population and 89% in high-risk patients. 57% considered alcohol consumption to be strongly associated with NAFLD. 60% deemed simple steatosis to confer excess liver-related mortality. 66% thought that NASH can be diagnosed with liver imaging. 71% made no referrals to hepatology services for suspected NAFLD.
Ratziu V, *et al.*, 2012 [12]	Survey assessing the clinical burden, perceived severity, and management patterns of NAFLD among 352, board-certified, hepato-gastroenterologists in France.	Most NAFLD patients were referred by GPs and only 20% by specialists. Conversely, 87% of hepatologists referred NAFLD patients for specialistic evaluation of potential co-morbidities. 65% would diagnose NASH irrespective of the concurrent CLD due to other etiology if MRFs were present. No agreement on the threshold of daily alcohol consumption that rules out NASH. Most physicians would overrate the importance of raised transaminases for the diagnosis of NASH. 62% delay LB after diet and lifestyle changes. 90% used non-invasive fibrosis markers. Roughy half did not measure fasting insulin/HOMA, 22% did not measure waist circumference. 73% monitored NAFLD patients themselves; most with yearly US and only 16% with fasting insulin/HOMA. 72% of patients were treated with non-pharmacological measures, often following referral to the endocrinologist/nutritionist. 42% recommended total abstinence from alcohol. Drugs treatment (metformin, UDCA, venesection, glitazones and vitamin E) was prescribed in only 28% of NAFLD patients.
Fishbein M, *et al.*, 2005 [15]	Analysis of physical examination findings and requests for diagnostic testing of 18 physicians involved in pediatric primary care on 11 obese children (4 with NAFLD) in USA.	Hepatomegaly was identified in 0.5% of obese children. Most commonly performed laboratory tests: fasting blood glucose (23%), lipid profile (20%), thyroid function tests (10%), and LTs (8.6%). Most common consultations: dietary (46%) and endocrinology (16%). Exercise program recommended in 4%. Abdominal imaging was requested in none of the encounters. In obese children with NAFLD, clinicians detected hepatomegaly in only 1.4% and requested LTs in 12.5% of encounters.
Sivertsen LM, *et al.*, 2008 [14]	Questionnaire assessing attitudes on diagnosis and management of overweight/obese children and awareness of clinical practice guidelines among 137 GPs in Australia.	The guidelines on the management of childhood obesity in general practice were reported to be used by 30% of respondents. 9% of GPs used BMI charts to correctly diagnose childhood obesity. 30% assessed for fatty liver in overweight/obese children. Over 80% of prescribed interventions were consistent with guidelines.
Riley MR, *et al.*, 2005 [16]	Retrospective chart review of 2256 pediatric outpatient visits at 2 academic hospitals (general pediatricians, pediatric endocrinologists and gastroenterologists) in USA.	Children with BMI 85 to 89%, 90 to 94% and ≥95% were given a diagnosis of overweight during 4, 8 and 48% of visits, respectively. General pediatrics, pediatric endocrinology and gastroenterology visits of overweight children included NAFLD screening in 2, 10 and 23% and metabolic screening in 8, 34 and 3% of cases, respectively.

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NAFLD in pediatrics was found (European Society for Pediatric Gastroenterology, Hepatology, and Nutrition [ESPGHAN]) [22].

The "real world" reports were analyzed to highlight the actual perception of NAFLD, verify the awareness of current recommendations, and identify the main difficulties experienced in clinical practice [8 16].

The recommendations issued by Scientific Societies were critically assessed in order to pinpoint the areas of convergence/difference.

The single position paper for pediatric medicine [22] was also examined in order to provide information useful to those involved in pediatric care.

Finally, prompted by the analysis of the reports of practicing physicians [8 16] and the systematic analysis/comparison of guidelines [17 22], we provide practical suggestions on NAFLD diagnosis and management in daily practice and highlight the open questions and future research.

Results and comments

Analysis of reports concerning issues from "real life" practice and selected guidelines disclosed the following major topics regarding NAFLD diagnosis and management that remain a matter of dispute (Tables 1 and 2):

(1) Definition and initial assessment of suspected NAFLD patients;
(2) Screening strategies for NAFLD;
(3) Diagnostic strategies: non invasive assessment and liver biopsy (LB);
(4) Management of NAFLD patients;
(5) Follow up strategies of NAFLD patients;
(6) Pediatric NAFLD.

What is the definition of NAFLD and which is the initial assessment of suspected NAFLD patients?

Analysis of reports from real life clinical practice

The single study evaluating the awareness of NAFLD in the general population demonstrated that the vast majority of people (83%) had never come across the term NAFLD; knowledge about NAFLD diagnosis and risk factors was also inadequate among those who had ever heard of it [13].

Similarly, several studies showed that knowledge about NAFLD diagnosis and assessment is relatively poor among GPs. An American study showed that GPs were less likely to consider NAFLD as a common cause of liver disease than Hepato Gastroenterologists [9]. These findings are consistent with an Italian survey: only 4.7% of GPs indicated a metabolic cause as the first determinant of an "undefined" persistent hypertransaminasemia. Moreover, a great variability in diagnostic approach to NAFLD was described [8]. In Loguercio's retrospective analysis involving 104 GPs, alcohol consumption, BMI, transaminases, and ultrasonography (US) were assessed only in a minority of patients with liver steatosis; no additional tests [markers of insulin resistance (IR), lipid profile, viral hepatitis serologies] were recorded [10].

In a recent survey of 100 hospital non liver specialists, >90% appreciated that traditional cardiovascular risk factors predicted NAFLD and acknowledged these to be common in non liver patients. Moreover, 57% considered alcohol consumption to be strongly associated with NAFLD [11].

A French survey among 352 Hepato Gastroenterologists showed that two thirds would diagnose NAFLD irrespective of the co existence of other CLD, as long as metabolic risk factors (MRFs) were present. There was no agreement on the threshold of daily alcohol consumption that ruled out the diagnosis of NAFLD. In the initial assessment of NAFLD patients, a large majority of surveyed specialists collected information on BMI, blood pressure, and glucose or lipid parameters; nonetheless, a sizeable proportion never assessed surrogate markers of IR or measurements of regional adiposity [12].

Analysis of guidelines

All guidelines agree that diagnosis of NAFLD relies on both imaging or histological evidence of hepatic steatosis and exclusion of causes of secondary hepatic fat accumulation; there is full agreement that NAFLD is strictly associated with MRFs. All Scientific Societies state that, because of the high prevalence of MRFs, NAFLD can co exist with other CLDs. There is universal consensus that the metabolic profile should be assessed, competing etiologies of steatosis and co existing CLD should be ruled out, and alcohol consumption should be estimated [17 21].

Regarding metabolic assessment, the majority of guidelines [17 20] highlight the importance of testing insulin sensitivity. However, there seems to be no consensus on how this should be done. All societies agree that presence of overweight/obesity should be evaluated through anthropometric measures (BMI, waist circumference) and that blood pressure and serum lipids measurement should be performed as a minimal initial assessment [17 21]. Regarding the criteria to adopt for the diagnosis of MS, the American guideline [21] recommends the Adult Treatment Panel III definition [23,24], whereas Asian Pacific Societies [18,19] recommend the International Diabetes Federation criteria [25].

All guidelines concur that all NAFLD patients should undergo a careful familial and medical history, viral hepatitis and autoimmune serology, alpha1 antitrypsin, iron and copper status measurement. The common association between chronic HCV infection and hepatic steatosis and its implications for fibrosis progression and/or treatment response rate are mentioned by all guidelines [17 21].

The threshold for hepatotoxic alcohol consumption to rule out alcoholic liver disease varies as a function of local drinking culture/habits. European Associations [17,20] maintain a threshold of 30 and 20 g of alcohol daily for men and women, respectively. Similarly, the American guideline [21] suggests 210/140 g (=21/14 drinks) of alcohol weekly, whereas Asian Pacific countries [18,19] restrict to 140/70 g of alcohol weekly for men and women, respectively. Moreover, the American guideline specifically recommends a 2 year alcohol withdrawal for NASH clinical trials candidate eligibility purposes [21]. This point is not discussed in other guidelines.

Comments

In recent years, the diagnostic strategy for NAFLD has evolved from a diagnosis of exclusion towards a *chiefly positive* approach based on the recognition of the underlying dysmetabolic milieu [1,2]. In patients with suspected NAFLD, exclusion of competing etiologies for steatosis is essential. To this end, endocrine disorders [26], familial hypobetalipoproteinemia [27], alcohol abuse,

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Table 2. Analysis of guidelines.

	AGA, AASLD, ACG [21]	CLDA [19]	IASL [20]	EASL [17]	APWP [18]	ESPGHAN [22]
Screening	-	+ (US and LTs in patients with MS)	/	+ (US and LTs in patients with MS and IR)	+ (US and LTs in patients with MS)	+ (US and LTs in overweight/obese children older than 3)
Initial evaluation						
Metabolic assessment	+	+	+	+	+	+
Competing causes of steatosis	+	+	+	+	+	+
Alcohol consumption	+ (M/F 21/14 drinks per wk)	+ (M/F 140/70 g weekly)	+ (M/F 30/20 g daily)	+ (M/F 30/20 g daily)	+ (M/F 140/70 g weekly)	/
Coexisting liver disease	+	+	+	+	+	+
Non-invasive assessment	+ (NAFLD Fibrosis Score)	- (only for research study)	+ (NAFLD Fibrosis Score and Fibroscan®)	+ (serum markers and Fibroscan®)	- (only for research study)	- (only for research study)
Liver biopsy	+ (restricted to selected patients)	+ (restricted to selected patients)	+ (restricted to selected patients)	+ (restricted to selected patients)	+ (restricted to selected patients)	+ (restricted to selected patients)
Management						/
Lifestyle intervention	+	+	+	+	+	
Pharmacological treatment	+ (pioglitazone and vitamin E in non-diabetic biopsy-proven NASH)	+	- (reserved to controlled studies)	+ (glitazones, vitamin E and high-dose UDCA in NASH)	- (reserved to controlled studies)	
Bariatric surgery	- (but is not contraindicated in eligible obese NAFLD)	+ (in obese patients refractory to medical measures)	- (reserved to controlled studies)	+ (in morbidly obese advanced fibrotic NASH)	+ (in obese patients refractory to medical measures)	
Metabolic control	+	+	+	+	+	
Follow-up						/
Hepatologic	+	+	+	+	+	
Cardiovascular	+	+	+	+	+	
Oncologic	-	+	+ (on individual basis)	-	+	
Children	Pediatric NAFLD section	/	/	/	/	Diagnostic aspects of pediatric NAFLD

+, recommended; -, not recommended; /, not mentioned.

and, particularly, HCV infection, given that HCV infection, diabetes and steatosis are closely linked to one another [28–30], need to be ruled out. Moreover, it is also necessary to carefully assess for MRFs and the cardiovascular risk profile. Furthermore, NAFLD can occur together with other CLD, which may accelerate the progression of liver injury [31–35]. Accordingly, in liver patients with MRFs, the presence of concurrent NAFLD should be evaluated. Conversely, when steatosis is detected in patients with CLD due to non NAFLD etiology, a metabolic assessment is needed. It is critical to define the appropriate standard anthropometric, biochemical and imaging protocol to be followed to detect NAFLD in clinical practice.

NAFLD definitely needs to be differentiated from alcoholic fatty liver disease (AFLD). However, due to the low reliability of the diagnostic methods (patient interview and biomarkers), a clear distinction between the two conditions is difficult [36–39]. Moreover, the recommended thresholds of "significant alcohol consumption" and the duration of alcohol withdrawal in those with suspected NAFLD are arbitrary. In addition, an overlap between alcohol consumption and metabolic disorders exists,

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making a clear attribution of steatosis to AFLD as opposed to NAFLD virtually impossible in the individual patient. For these reasons, some authors consider this distinction of fatty liver dis ease artificial and poorly useful [40].

Key Points 1

- Awareness of NAFLD, its diagnosis, and risk factors in the general population is poor. Knowledge about NAFLD diagnosis and assessment is relatively inadequate among general practitioners, particularly so in NAFLD pediatric patients. Specialists other than hepatologists under-appreciate the overlap between NAFLD and metabolic risk factors, thus missing a significant proportion of high-risk NAFLD patients. Hepatologists themselves risk under-diagnosing NAFLD due to over-reliance on transaminases

Who and how to screen for NAFLD?

Analysis of reports from real life clinical practice

Grattagliano's survey showed that 70% of Italian GPs underesti mated the prevalence of NAFLD among the general adult popula tion, only 36.6% would screen for NAFLD diabetic subjects and a substantial subset of hypertransaminasemic patients were not considered for NAFLD even in the presence of MRFs. Specific training significantly improved GPs' ability in screening at risk patients [8]. The underestimation of the NAFLD problem by GPs was confirmed by another Italian study, in which an extremely low prevalence of fatty liver was reported, and a high proportion of patients were considered as affected by "undefined" CLD despite a high rate of overweight/obesity and an incomplete diag nostic work up [10]. GPs are reported to be less familiar with cur rent recommendations and to use appropriate screening strategies less frequently than hepato gastroenterologists unless they are fully aware of guidelines [9].

An Australian survey showed that also non liver Specialists underestimated the prevalence of NAFLD both in the general pop ulation and in high risk patients, thus reflecting a low grade of referrals to Hepatology services [11]. Accordingly, a French study reported that only 20% of NAFLD patients seen in gastroenterol ogy practice were referred by specialists in the metabolic field. This survey stressed that among liver specialists there was an over reliance on transaminases instead of MRFs or US steatosis, when considering the diagnosis of NAFLD [12].

Analysis of guidelines

The majority of guidelines [17 19] explicitly suggest the opportunity to implement a screening policy in individuals at high risk of NAFLD identified by the presence of MRFs and/or IR. Two guidelines either fail to mention [20] or discourage any screening policies [21]. Indeed, the most recent American guideline [21] states that systematic screening for NAFLD is not recommended not only in the general population but also in high risk patients, in family members and in obese children, due to paucity of evidence.

All Scientific Societies who support screening suggest that it should be done through both US and Liver Tests (LTs).

Comments

The prevalence of NAFLD in the general population ranges from 6.3% to 51% depending on the method used to assess liver steatosis and the population/ethnicity studied [41 45]. This prev alence can be significantly higher in individuals with MRFs [46 48]. Moreover, familial aggregation and heritability of NAFLD have been consistently reported [49 52].

There are important differences concerning the definitions of overweight/obesity and MS between Western and Asia Pacific patients. In the Asian population, morbidity and mortality occur at lower BMIs and smaller waist circumferences than in Cauca sians, justifying specific criteria for overweight/obesity and MS representative of people living in the Asia Pacific region [25,53 55].

Although the majority of NAFLD cases are strongly associ ated with overweight/obesity and T2D, different studies reported a prevalence of NAFLD in the normal weight popula tion between 7% and 16% [42,56 59]. These studies invariably demonstrated that NAFLD is closely associated with metabolic disorders, particularly IR, even in lean patients. NAFLD should be considered an early predictor of metabolic derangements, thus suggesting that IR, rather than frank diabetes or obesity, is the alteration to be detected when screening for NAFLD. Therefore, methods and thresholds to define subtle IR are strongly needed in order to detect those patients at increased risk of hepatic complications.

Compared to the general population, NAFLD is independently associated with a significantly higher all cause mortality [5 7,60 65], and cancer incidence [66,67], principally HCC [68,69], increased incident T2D risk [6,70,71], greater prevalence/inci dence of cardiovascular disease (CVD) [72 75], and a higher rate of major complications and death after surgery [76 78].

Based on the above reasons, detection of NAFLD should be considered as a major task in the management of patients with features of IR. Nevertheless, due to uncertainties surrounding the best diagnostic and management strategy, unequivocal indi cations in NAFLD screening policies are lacking.

US, being safe, inexpensive, widely available, and having a good performance when steatosis is present in at least 20 30% of hepatocytes is an acceptable first line screening proce dure for NAFLD in clinical practice. However, the relatively low acuity for mild steatosis, the low accuracy in morbid obes ity, and its operator dependency are the main limitations [79,80]. Interestingly, although not so sensitive as magnetic resonance spectroscopy [81,82], US can nevertheless have a lower threshold for fat detection than previously appreciated [80]. Criteria used to define US steatosis need to be standard ized and semi quantitated. Once such semi quantitation is per formed through simple scores, US is able to predict metabolic derangements and liver histology changes [83,84].

Despite the almost universal reliance on transaminases in real life practice, LTs are not considered a useful tool in NAFLD screening. Indeed, the majority of NAFLD patients have normal transaminases [42], which do not rule out histologically advanced disease [85,86]. The definition of the "normal" trans aminases range is controversial. Transaminases reference ranges currently used underestimate the prevalence of patients with liver diseases and the upper limit of "normal" alanine aminotransferase has been downgraded to 30 U/L for men and 19 U/L for women [87 92].

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Key Points 2

- All guidelines agree that the diagnosis of NAFLD relies on both imaging and histological evidence of hepatic steatosis after exclusion of competing etiologies of liver fat deposition (typically HCV infection, alcohol consumption and other) in individuals with metabolic risk factors. NAFLD patients should undergo a careful familial and medical history, viral hepatitis and autoimmune serology, alpha 1-antitrypsin, iron and copper status measurement. The threshold for hepatotoxic alcohol consumption and the extent of alcohol withdrawal to rule out alcoholic liver disease remain to be defined. NAFLD may well co-exist with other chronic liver diseases, typically HCV infection. The majority of guidelines suggest the opportunity to implement a screening policy (through both US and LTs) in individuals at high risk of NAFLD identified by the presence of metabolic risk factors and/or IR. LB should not be performed in all NAFLD patients but should be restricted to those NAFLD patients presenting an increased risk for NASH or advanced fibrosis

How to non-invasively assess inflammation and fibrosis and when to obtain an LB?

Analysis of reports from real life clinical practice

Grattagliano reported that the majority of GPs indicated hyper transaminasemia or none as the best reason to ask for LB in NAFLD subjects. Only 2.3% of GPs chose over 50 year old diabetic patients as potential candidates for LB. However, after attending a tailored workshop, 80.9% indicated the latter as good candidates for LB and a substantial proportion reconsidered a fraction of their previously diagnosed NAFLD patients at potential risk of NASH [8].

The Australian survey among non liver Specialists reported that 98% correctly identified that NASH can be diagnosed on LB, about three quarters agreed that LTs are not sufficiently sensitive to detect NASH, but 66% deemed that a diagnosis of NASH can be based on imaging [11].

Ratziu showed that about two thirds of Hepato Gastroente rologists considered important the identification of steatohepati tis or the staging of fibrosis. However, the main indication for LB was to gauge the fibrosis stage. In fact, given the invasive nature of LB, 38% would not perform this procedure to estimate hepatic inflammation. Confirming that transaminases levels impact on the decision to perform an LB, 43% of hypertransaminasemic *vs.* 6% of normotransaminasemic NAFLD patients would be asked to undergo an LB. Non invasive fibrosis markers were used by 90% of the surveyed physicians in clinical practice: the majority used both serum markers and elastometry [12].

Analysis of guidelines

Initial non invasive assessment of inflammation and fibrosis is suggested in clinical practice by some [17,20,21] but not all guide lines. CLDA and APWP restrict non invasive assessment of NASH and fibrosis to research purposes alone [18,19]. European and Ital ian guidelines suggest the combined use of clinical and laboratory parameters, serum markers, composite scores (particularly the NAFLD fibrosis score) and imaging methods (transient elastogra phy FibroScan) in order to reduce the number of NAFLD patients requiring LB [17,20]. The American guideline confirms the clinical utility of NAFLD fibrosis score in identifying NAFLD patients with higher likelihood of having advanced fibrosis and highlights the importance of MS as strong predictor of NASH [21].

There is universal agreement that LB should not be performed in all patients. All guidelines recommend LB in NAFLD patients presenting an increased risk for NASH or advanced fibrosis [17 21]. LB is considered in suspected NAFLD patients in whom there is diagnostic uncertainty due to difficulties in excluding compet ing etiologies for hepatic steatosis and co existing CLD by the majority of guidelines [18,19,21]. The European guideline recom mends performing LB to assess concurrent NAFLD in patients with other CLD, MRFs, and US steatosis [17]. Asian Pacific and European guidelines suggest the opportunity to perform LB in NAFLD patients subjected to surgical procedures for other pur poses [17,18]. All guidelines (implicitly or explicitly) recommend LB in NALFD patients enrolled in clinical trials [17 21].

Comments

Simple steatosis is associated with a normal life expectancy and its progression is limited to anecdotal case reports [93 95]. Con versely, NASH worsens in up to 30% of cases, evolving in cirrhosis in a substantial fraction of cases [3,61,96]. Moreover, 30 75% of cases of cryptogenic cirrhosis can be attributed to previously unrecognized NASH [68,97 101]. Given that the presence of inflammation at the initial LB is the strongest predictor of NAFLD progression and that the degree of fibrosis is the most important prognostic factor, efforts of practicing physicians should be ori ented towards identification of those patients with steatohepatitis and/or advanced fibrosis.

LB is the gold standard for direct diagnosis of NASH and eval uation of inflammation/fibrosis, however, its use is limited by invasiveness, cost and sampling error [102]. Several non invasive methods for identifying patients with NASH or fibrosis have been proposed [5,103 106], but validated decisional algorithms ade quate for clinical practice are still lacking.

Key Points 3

- All NAFLD patients should undergo interventions aimed at promoting healthier lifestyles and strict control of metabolic risk factors associated with NAFLD. Pharmacotherapy (glitazones, vitamin E, possibly associated with high-dose UDCA) should be reserved for NASH patients possibly in randomized controlled trials. Concurrent metabolic risk factors associated with NAFLD should be managed as clinically required and drugs given as needed. Bariatric surgery, if otherwise indicated, is considered a valid option for obese patients with NAFLD/NASH. Heavy alcohol consumption should be discouraged. Light-moderate alcohol consumption may exert favorable metabolic effects and, perhaps, on liver outcomes. However, in the absence of randomized controlled trials, all guidelines advise against prescribing low-moderate alcohol consumption as a preventive/therapeutic strategy fort NAFLD. Hepatological and cardiovascular follow-up is indicated in NAFLD patients. Oncologic screening/surveillance should be considered on individual risk

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How to treat NAFLD patients?

Analysis of reports from real life clinical practice

In the Italian survey, 78% and 91% of GPs, before and after the workshop, respectively, indicated diet as the first and best approach to NAFLD. 34.1% stated that statins should be avoided in NAFLD patients [8].

In Bergqvist's study, 95% of non hepatologists agreed that management of NAFLD involves weight loss, physical exercise, and treatment of concurrent MRFs. Further to lifestyle changes, drugs to lose weight and bariatric surgery were included in NAFLD management, whereas 75% of respondents excluded specific liver directed drug therapy [11].

The French survey among hepatologists showed that 72% of patients were treated with lifestyle changes only, while 28% were treated with drugs further to non pharmacological interventions. The most frequently prescribed regimens were: metformin, ursodesoxycholic acid, phlebotomy, glitazones, and vitamin E. 42% recommended total abstinence from alcohol; about 50% allowed daily alcohol consumption of 10–30 g in male and 10–20 g in female patients [12].

Analysis of guidelines (Table 3)

There is universal consensus that all patients should undergo interventions aimed at promoting healthier lifestyles and strict control of MRFs associated with NAFLD. All guidelines agree that lifestyle changes including weight loss, dietary changes, and physical exercise should always be implemented as first line option in all NAFLD patients [17–21].

With regard to the entity of weight loss, the Italian guideline simply states that 0.5 kg/week weight loss should be considered in overweight individuals [20], whereas the Chinese guideline recommends more than 5% weight reduction in 6–12 months [19]. The European guideline suggests that a weight loss of 7% should be reasonable in overweight and mildly obese patients [17]. Finally, American societies provide more specific indications: loss of at least 3–5% of body weight to improve steatosis, and up to 10% to improve necroinflammation [21].

All societies concur in recommending a hypocaloric diet to promote weight loss [17–21]. However, while the Chinese guideline provides quantitative details (intake of 500–1000 kcal daily for obese adults) [19], almost all guidelines identify qualitative directions (low carbohydrate and saturated fat intake, avoidance of fructose enriched soft drinks and increased consumption of fibers and antioxidants rich fruits and vegetables) [17,19,20].

All guidelines agree that heavy alcohol consumption should be avoided in NAFLD patients. However, no guidelines encourage mild–moderate intake [17–21].

All guidelines strongly recommend avoidance of sedentariness and implementation of physical activity. The European guideline is more accurate in suggesting at least 150 min per week of moderate intensity physical activity and at least 75 min per week of vigorous intensity physical activity, further to muscle strengthening twice a week [17]. Similarly, the Chinese guideline recommends moderate aerobic exercise at least 4 times weekly, with a minimum cumulated exercise time of 150 min [19]. Moreover, European societies [17,20] and the Chinese guideline [19] highlight behavior therapy as important in accomplishing weight loss.

Pharmacologic therapy should be reserved only to NASH. The more conservative suggestion is to limit the use of drugs to randomized controlled trials [18,20]. However, EASL suggests a 1–2 year course of therapy with glitazones or vitamin E, preferably associated with high dose UDCA [17]; the AGA–AASLD–ACG guideline advocates pioglitazone and vitamin E in non diabetic biopsy proven NASH [21]; and the Chinese guideline proposes liver protective and anti inflammatory drugs, including Chinese traditional and western medicines, in biopsy proven NASH [19].

All guidelines agree that the underlying MRFs should be managed as clinically required in NAFLD patients and drugs (particularly statins for dyslipidemia) given as needed [17–21]. Bariatric surgery, if otherwise indicated, is considered a valid option for obese patients with NAFLD/NASH by all but one guideline [20].

Comments

The management of NAFLD patients is based on treatment of liver disease alongside the associated MRFs [107,108]. Data on this topic are many and perhaps confusing. Guidelines are influenced by the year of publication. There are no medications specifically approved for NASH, therefore drug treatments specifically aimed at liver disease should be reserved to randomized trials with histological end points. Interestingly, there is increasing evidence for a beneficial effect of pioglitazone and vitamin E on liver outcomes in non diabetic patients with biopsy proven NASH [109], and a recent cost–utility analysis indicated that, in subjects with NASH and advanced fibrosis, treatment with either pioglitazone or vitamin E further to standard lifestyle changes is likely cost effective [110]. However, pioglitazone, vitamin E, and UDCA are not free of side and toxic effects. Pioglitazone is associated with weight gain and an increased risk of congestive heart failure, bone fractures, and bladder cancer [111,112]. High dose vitamin E has been linked to increased all cause mortality and an excess hemorrhagic stroke and prostate cancer [113,114]. High dose UDCA determines diarrhea and abdominal discomfort [115].

From a practical perspective, ameliorating cardiometabolic risk profile and histological disease activity, lifestyle induced weight loss should be recommended in all NAFLD patients, but clear targets and suggestions on how to reach them are needed. It should be highlighted that the common pharmacological treatment of MRFs (particularly statins) is not contraindicated in NAFLD [116].

As far as alcohol intake concerns, on the one hand, heavy consumption is harmful to the liver [117] and should be discouraged. On the other hand, light–moderate alcohol consumption might well exert favorable effects on MRFs and, perhaps, on liver outcomes [118–121]. However, in the absence of randomized controlled trials, all guidelines discourage from prescribing low–moderate alcohol consumption as preventive/therapeutic strategy against NAFLD.

How to follow-up NAFLD patients?

Analysis of reports from real life clinical practice

Two thirds and 22% of the surveyed Australian non liver Specialists considered semi annual LTs and 5 yearly LB as the most effective method for monitoring NAFLD patients [11]. The majority of French Hepatologists stated to monitor their NAFLD

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Table 3. How to manage NAFLD patients?

	AGA, AASLD, ACG [21]	CLDA [19]	IASL [20]	EASL [17]	APWP [18]
Weight loss	+ (3-5% to improve steatosis, 10% to improve NASH)	+ (more than 5%)	+ (0.5 Kg/wk)	+ (7%)	+
Hypocaloric diet	+	+ (500-1000 Kcal)	+	+	+
Alcohol	-,?	-	- (particularly in obese NAFLD)	?	/
Physical exercise	+	+ (4 times per week, 150 min of aerobic exercise)	+	+ (150 min/wk moderate and 75 min/wk vigorous exercise)	+
Educational therapy	/	+	+	+	/
Metformin	-	- (not contraindicated in diabetic NAFLD)	- (not contraindicated in diabetic NAFLD)	- (not contraindicated in diabetic NAFLD)	- (not contraindicated in diabetic NAFLD)
Glitazones	+ (pioglitazone in non-diabetic biopsy-proven NASH)	- (not contraindicated in diabetic NAFLD)	- (not contraindicated in diabetic NAFLD)	+ (NASH)	- (not contraindicated in diabetic NAFLD)
Vitamin E	+ (in non-diabetic biopsy-proven NASH)	+	-	+ (NASH)	-
UDCA	-	+	-	+ (NASH)	-
Omega-3 FA	- (not contraindicated in hypertriglyceridemic NAFLD)	+	- (not contraindicated in hypertriglyceridemic NAFLD)	- (not contraindicated in hypertriglyceridemic NAFLD)	- (not contraindicated in hypertriglyceridemic NAFLD)
Statins	- (not contraindicated in dyslipidemic NAFLD)	- (not contraindicated in dyslipidemic NAFLD)	- (not contraindicated in dyslipidemic NAFLD)	- (not contraindicated in dyslipidemic NAFLD)	- (not contraindicated in dyslipidemic NAFLD)
Bariatric surgery	- (not contraindicated in eligible obese NAFLD)	+ (in obese patients refractory to medical measures)	- (reserved to controlled studies)	+ (in morbidly obese advanced fibrotic NASH)	+ (in obese patients refractory to medical measures)

+, recommended; –, not recommended; /, not mentioned.

patients with a mean number of two annual visits. LTs and US were the most frequently performed procedures. 57% did not per form follow up LB. With regards to MRFs, the majority of sur veyed specialists monitored glycemic and lipid profile, and half of those who assessed these parameters did so twice a year. How ever, surrogate markers of IR were never monitored by at least 50% [12].

Analysis of guidelines

There is universal consensus on the opportunity to perform hepatological and cardiovascular follow up in NAFLD patients [17 21]. In NAFLD patients, semi annual to annual hepatic mon itoring (non invasive follow up of fibrosis, liver US, transami nases and LTs, markers of IR) is warranted [17,19]. Routine repetition of LB is not indicated [21]. LB may be repeated not earlier than 5 years after baseline LB in those patients in whom fibrosis progression is suspected [17]. Surveillance for eso phago gastric varices [17,19,21] and HCC [17,19 21] in patients with NASH cirrhosis is advocated by the majority of societies.

All societies agree that a thorough assessment of MRFs and a risk stratification for CVD should be done in all NAFLD patients [17 21]. These evaluations should be repeated every 6 months 1 or 2 years [17,19,20]; the interval between check ups should be modulated on an individual basis, mirroring the severity of liver disease and clustering of MRFs [17,20]. Generalized cancer screening programs cannot be proposed to all NAFLD patients [20]. Three out of five guidelines support the practice of oncologic

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follow up on individual basis [18 20]. Four scientific societies specifically mention HCC among the cancer types to which NAFLD patients may be prone [17,19 21]. The guideline of the Asia Pacific region suggests to extend screening to those "*cancers whose incidence is increased by MS*" [18].

Comments

Considering the natural history of NAFLD, in terms of liver related, metabolic, cardiovascular and neoplastic complications, patients affected warrant screening for MRFs and progressive liver disease [5]. However, most of our understanding of the natural course of hepatic and extrahepatic co morbidities of NAFLD is based on data from hepatological referral centers evaluating selected groups of individuals [78]. Despite such limitations, the increasing burden of NAFLD related primary liver cancers, principally HCCs [68,69] that may occur in non cirrhotic NAFLD [122], suggests the opportunity of more liberal surveillance programs in these patients. However, specific rec ommendations about screening for HCC in NAFLD patients are lacking and there are no data on the cost effectiveness of sur veillance programs in these patients. Moreover, an increased risk of colorectal and other types of cancers has been described in NAFLD patients [66,67]. Efforts should be made to identify the cardiometabolic, hepatologic, and oncologic risks in the individual patient and to develop personally tailored follow up schedules.

Key Points 4

More attention should be paid to medical education and emphasis be placed in integrated NAFLD management. Questions to be answered are:

- The definition of NAFLD natural history in the general population rather than in cohorts selected in tertiary referral centers,
- The definition of unequivocal NAFLD screening policies,
- The assessment of methods and thresholds to define subtle IR,
- The validation of decisional algorithms for LB submission,
- The identification of methods to obtain healthy life-style changes targets,
- The definition of personally-tailored cardiometabolic, hepatologic, and oncologic surveillance strategies

Pediatric NAFLD

Analysis of reports from real life clinical practice

A survey among American primary pediatric care providers showed that, in obese children with NAFLD, clinicians detected hepatomegaly in only 1.4% and requested LTs in 12.5% of encoun ters, thus increasing the likelihood of a delayed or omitted diag nosis [15]. An Australian survey described that only 9% of GPs used BMI charts to correctly diagnose childhood obesity and only 30% assessed for fatty liver in overweight/obese children [14].

Another survey among general pediatricians and pediatric endocrinologists and gastroenterologists at two American aca demic hospitals confirmed the underdiagnosis of obesity and the underscreening for MS and NAFLD in children [16].

Analysis of guidelines

Among adult NAFLD guidelines, only the American one deals with specific aspects of pediatric NAFLD [21]; a single position paper is specifically devoted to diagnosis of NAFLD in children and adoles cents [22]. The American guideline and ESPGHAN statement dis agree with regard to screening for NAFLD in overweight/obese children. American societies suggest that a formal recommenda tion cannot be made [21], whereas ESPGHAN states that NAFLD should be suspected in all overweight/obese children and adoles cents older than 3 years especially if familiarity for NAFLD is present [22].

According to ESPGHAN, abdominal US and LTs should be the first diagnostic step in suspected NAFLD children, followed by exclusion of other liver diseases [22]. The two guidelines agree that very young or lean children with liver steatosis should be tested for monogenic metabolic disorders as causes of fatty liver [21,22].

Both documents suggest similar indications for LB: to rule out other treatable diseases, in cases of clinically suspected advanced liver disease, before pharmacological/surgical treatment, and as part of a structured intervention protocol or clinical research trial [21,22]. Only the American guideline discusses treatment of pedi atric NAFLD. According to AGA AASLD ACG, intensive lifestyle modification is recommended as the first line treatment in pedi atric NAFLD. Metformin should be avoided. Vitamin E offers his tological benefits to children with NASH, but confirmatory studies are needed before its use can be recommended in clinical practice [21].

Comments

The rising incidence of obesity is paralleled by the increasing rec ognition of NAFLD also in children and adolescents [123,124]. Due to its potential progressive nature also in childhood [125,126], early diagnosis and treatment are important in all age groups [127]. Therefore, shared standards to be used by phy sicians caring pediatric NAFLD are needed. Non invasive diagnos tic strategy represents a key issue in pediatric practice. However, contrasting with adult medicine, relatively scarce data are avail able in pediatric patients [105,128].

Discussion

Given that NAFLD epidemic poses a heavy health related costs burden [129], an effort is justified to improve our medical ability in clinical practice. A successful management plan requires a motivated public, competent primary care doctors and special ists, and the implementation of multidisciplinary collaborative networks [130]. However, studies in "real life" practice have shown that: (1) awareness of NAFLD is low in the general popu lation [13]; (2) knowledge of NAFLD and its complications is not properly diffused among GPs who thus may fail to approach some

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Table 4. Open questions and future studies.

Screening	• Which screening/surveillance policies for NAFLD in individuals with a single MRF and incomplete features of the MS (e.g. T2D/obesity/dyslipidemia/hypertension alone or variedly associated)? • What about screening in "NAFLD families"? • What about screening for NAFLD in the setting of liver transplantation and major hepatic surgery? • New lowered aminotransferases ranges?
Initial evaluation	• Which is the appropriate standard anthropometric, biochemical and imaging protocol to be followed to detect NAFLD in clinical practice? • Definition of threshold and duration of alcohol consumption • How to measure IR? Which ranges? • A role for genetics [131]? • A role for gut microbiota [132-134]?
Non-invasive assessment	• NASH and fatty liver: two different entities? • Which diagnostic protocols/algorithms in clinical practice? • A role for novel US scoring systems [83,84]?
Liver biopsy	• Which criteria to restrict the number of individuals to submit to LB through strict pre-biopsy testing? • More liberal use in those undergoing surgery for related conditions: gallstones, T2D, obesity? • Is LB mandatory in clinical trials (when are surrogate indices enough?)?
Management	• Diets: standard criteria *vs.* general suggestions? • Entity and types of physical activity? • Role of psychological support-web-based platforms? • Alcohol intake: pros and cons? • When are drugs indicated? • Iron depletion: when, why and how [135,136]? • Gut microbiota: a role for probiotics/antibiotics?
Follow-up	• Definition of NAFLD natural history in unselected populations • Cost-effective analysis of personally tailored screening/surveillance programs for liver-related, cardiovascular and oncologic complications
Children	• Non-invasive diagnostic strategy of NAFLD in pediatric age • To extend recommendations for NAFLD in adults to children *vs.* specific recommendations for pediatric NAFLD?

aspects of diagnosis and management [8 10,14,15]; (3) specialists other than hepatologists may miss a high proportion of high risk NAFLD patients and under appreciate the overlap between NAFLD and other MRFs [11,12,16]; (4) a proportion of hepatologists risk to under diagnose NALFD due to over reliance on transaminases [12].

Taken collectively, some data [8 16] support that more attention should be paid to medical education and emphasis be placed in integrated NAFLD management. Indeed, awareness of guidelines and teaching programs consistently improve specific competence of practicing physicians [8,9]. Moreover, increased consistency among guidelines issued by different medical societies might eventually result in improved care of NAFLD in clinical practice [9].

Here we have raised awareness of existing guidelines for NAFLD and provided practical suggestions on the chief controversial topics regarding diagnosis and management of NAFLD in daily practice.

In summary:

(1) A new *positive* definition of NAFLD, in which IR and MRFs are the mainstay, is required. All guidelines agree that patients suspected for NAFLD should undergo, as the initial evaluation, a careful assessment of MRFs, competing causes of liver steatosis (particularly HCV infection, alcohol abuse and other), and co existent CLD [17 21].

(2) Screening for NAFLD is not recommended in the general population. All but one guideline [21] recommend screening for liver steatosis in patients with MRFs. Features of IR should be considered a major prompt to detect NAFLD. US should be the first line screening procedure for NAFLD [79,80], whereas transaminases are not a useful tool in clinical practice [85,86].

(3) Non invasive tests are needed to predict NASH and fibrosis in NAFLD patients in order to restrict LB to selected individuals. NAFLD fibrosis score and FibroScan could be useful to this end [5,105]. LB is universally considered the diagnostic and prognostic standard in NAFLD. However, given its invasiveness and costs, there is full agreement in limiting its use on a case by case basis.

(4) All guidelines agree that lifestyle modifications are the first line approach to manage NAFLD patients [17 21]. Bariatric surgery could be a valid option in morbidly obese NAFLD patients non responders to lifestyle changes. Pharmacologic therapy should be restricted to clinical trials. Specific drug treatments of MRFs (particularly statins) are not contra indicated in NAFLD patients.

(5) NAFLD patients should undergo regular follow up not only for liver related complications but also for metabolic and cardiovascular diseases. Oncologic screening/surveillance should be considered on individual risk.

(6) Pediatric NAFLD shares the same MRFs as NAFLD in adults. Diagnosis of NAFLD in children requires a thorough work up and exclusion of age specific diagnoses.

In conclusion, current guidelines appear to be somewhat heterogeneous, if not contradictory, and fragmentary, suggesting the opportunity to implement global recommendations concerning the conduct to be followed in real life clinical practice and much

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research remains to be done about NAFLD screening, diagnosis, management, and follow up (Table 4).

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Conflict of interest

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Article 2. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. Aliment Pharmacol Ther. 2014 ; 40 : 1209 - 1222

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Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease

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SUMMARY

Background
Several steatosis biomarkers are available with limited independent validation.

Aim
To determine diagnostic value and limitations of several steatosis biomarkers using liver biopsy as reference standard in a large cohort of patients with suspected NAFLD.

Methods
Three hundred and twenty-four consecutive liver biopsies were included. Histological steatosis was categorised as none (<5%), mild (5–33%), moderate (33–66%) and severe (>66%). Five steatosis biomarkers were measured: fatty liver index (FLI), NAFLD liver fat score (NAFLD-LFS), hepatic steatosis index (HSI), visceral adiposity index (VAI) and triglyceride × glucose (TyG) index.

Results
Steatosis grades prevalence was: none 5%, mild 39%, moderate 30% and severe 27%. Except for VAI, the steatosis biomarkers showed a linear trend across the steatosis grades. However, their correlation with the histological amount of steatosis was only weak-moderate. All steatosis biomarkers had an adequate diagnostic accuracy for the presence of steatosis: AUROCs for FLI, LFS, HSI, VAI and TyG were 0.83, 0.80, 0.81, 0.92 and 0.90. However, their ability to quantify steatosis was poor: none of them distinguished between moderate and severe steatosis and the AUROCs for predicting steatosis >33% were 0.65, 0.72, 0.65, 0.59 and 0.59 for FLI, LFS, HSI, VAI and TyG. Both fibrosis and inflammation significantly confounded the association between steatosis biomarkers and steatosis. The steatosis biomarkers were all correlated with HOMA-IR, independent from histological steatosis.

Conclusions
All five steatosis biomarkers can diagnose steatosis and are correlated with insulin resistance. They are confounded by fibrosis and inflammation, and do not accurately quantify steatosis; this may limit their clinical utility. More research is needed to identify truly independent and quantitative markers of steatosis.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), has an estimated prevalence of 20–30% in the general population and still higher in individuals with metabolic risk factors.[1–3] It is currently a leading cause of chronic liver disease and could become an important health concern because of associated hepatic and extrahepatic morbidity and mortality.[4, 5]

Liver ultrasonography, a largely available procedure for first-line fatty liver detection in clinical practice, has a low sensitivity as it only detects steatosis when present in more than 20–30% of hepatocytes.[6, 7] Besides, it cannot reliably quantify steatosis, an important drawback for the ability to monitor dietary and pharmacological interventions. Magnetic resonance spectroscopy is a highly sensitive and quantitative imaging procedure, but cost and availability severely restrict its use in clinical practice. Liver biopsy is therefore still the most sensitive tool for diagnosing and monitoring changes in liver fat. Nevertheless, it is an invasive and costly procedure not suitable for screening or monitoring. Hence, there is a considerable interest in developing noninvasive, simple, accurate and cost-effective methods for early identification and quantification of steatosis in the setting of screening, follow-up and evaluation of treatment.

Several noncommercial steatosis biomarkers based on aggregate scores from different anthropometric and metabolic parameters have been published.[8–11] Independent validation of these steatosis biomarkers is very scarce. Because of substantial heterogeneity in the types of patients included such an independent validation is critical before recommending these tests for wider clinical practice or research purposes. The fatty liver index (FLI) has shown good performance in detecting fatty liver in several population studies; however, it has only been validated against liver ultrasonography.[9, 12–14] The hepatic steatosis index (HSI), derived from data of a Korean cross-sectional case–control study involving more than 10 000 patients, is a simple and promising score for predicting NAFLD, but it has been validated against ultrasound and lacks external validation.[11] The NAFLD-LFS has been validated against magnetic resonance spectroscopy showing an overall good accuracy in diagnosing NAFLD; however, it has limited external validation.[10, 15] The VAI, a surrogate biomarker of visceral adiposity,[16] and the Triglycerides × Glucose (TyG) index,[17] an indirect score of insulin resistance, have been both demonstrated to be independently associated with histologically defined steatosis in patients with chronic hepatitis C.[18, 19] However, whether VAI predicts liver histology in patients with NAFLD is controversial and, to our knowledge, TyG index has never been specifically investigated in NAFLD.[20–22]

In this study, we aimed to determine the diagnostic performance of the five above-mentioned biomarkers (FLI, NAFLD-LFS, HSI, VAI and TyG), including their ability to quantitatively predict the amount of steatosis, in a large cohort of biopsy-proven NAFLD patients.

PATIENTS AND METHODS

Study population and measurements

We retrospectively analysed 324 consecutive liver biopsies performed between 2000 and 2010 for clinical and/or ultrasonographic suspicion of NAFLD. Exclusion criteria were: alcohol consumption ≥30 g/day in men or ≥20 g/day in women, presence of hepatitis B surface antigen or anti-hepatitis C virus antibodies, genetic hemochromatosis, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, alpha1-antitrypsin deficiency, Wilson's disease, drug-induced liver disease, cardiac insufficiency or any other chronic liver disease that could coexist in addition to NAFLD. Patients taking medications that can induce secondary NASH (corticosteroids, amiodarone, tamoxifen) were not included.

Clinical, anthropometric, laboratory and ultrasonographic data were collected within a 6 month time-period from the date of liver biopsy. A complete medical history and physical examination was undertaken in all patients. All patients underwent measurements of blood pressure (BP), weight, height and waist circumference (WC) according to uniform protocols. Body mass index (BMI) was calculated using the standard formula: weight (kg)/height^2 (m^2). Laboratory evaluation included routine liver biochemistry, lipid profile [total cholesterol, high-density lipoprotein cholesterol (HDLc) and total triglycerides], fasting glucose and insulin. The degree of insulin resistance was determined by the homoeostatic model assessment (HOMA-IR) using the formula: (insulin × glucose)/22.5.[23] Liver ultrasonography data for the assessment of hepatic steatosis (bright liver pattern with liver/kidney gradient) were also retrieved.

The metabolic syndrome was defined according to criteria of the International Diabetes Federation[24]: central obesity (WC ≥94 cm in men and ≥80 cm in women)

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and at least two other metabolic alterations among serum triglycerides ≥1.70 mmol/L or specific treatment for hypertriglyceridemia, serum HDLc <1.03 mmol/L in men and <1.29 mmol/L in women or specific treatment for this lipid abnormality, systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg or treatment for previously diagnosed hypertension, and fasting plasma glucose ≥5.6 mmol/L or previously diagnosed type 2 diabetes.

Histological assessment

Liver biopsies were performed percutaneously under ultrasound guidance. All liver biopsies were adequate in terms of length, absence of fragmentation and quality of staining. Liver biopsies were routinely formalin-fixed, paraffin-embedded and haematoxylin & eosin and Picrosirius Hemalun stained. All slides were read by a single expert liver pathologist (F.C.), blinded to clinical data, and categorised using the scoring system proposed by Kleiner *et al.*[25] Steatosis was categorised as none if the presence of steatosis was less than 5%, mild (≥5–33%), moderate (>33–66%) and severe (>66%). We defined nonalcoholic steatohepatitis (NASH) as the presence of at least 5% of steatosis with at least both grade 1 hepatocellular ballooning and lobular inflammation following a mainly centrilobular pattern of distribution. Mild fibrosis was defined as F0–F2 and advanced fibrosis as F3–F4 (i.e. bridging fibrosis and cirrhosis). Advanced NASH was empirically defined as the simultaneous presence of moderate/severe steatosis (>33%), NASH and advanced fibrosis.

Steatosis biomarkers

Five liver steatosis biomarkers, FLI, NAFLD-LFS, HSI, VAI and TyG index, were calculated using the clinical, anthropometric and laboratory data retrieved at the time of each liver biopsy, according to the following formulas:

$$\text{FLI} : [e^{0.953 * \log e\,(\text{triglycerides}) + 0.139 * \text{BMI} + 0.718 * \log e(\text{GGT}) + 0.053 * \text{WC} \quad 15.745}] / [1 + e^{0.953 * \log e(\text{triglycerides}) + 0.139 * \text{BMI} + 0.718 * \log e\,(\text{GGT}) + 0.053 * \text{WC} \quad 15.745}] * 100.^{9}$$

$$\text{NAFLD-LFS} : -2.89 + 1.18 * \text{metabolic syndrome}(\text{yes} = 1/\text{no} = 0) + 0.45 * \text{type 2 diabetes}\ (\text{yes} = 2/\text{no} = 0) + 0.15 * \text{insulin}(\text{mU/L}) + 0.04 * \text{AST}(\text{U/L}) - 0.94 * \text{AST/ALT}.^{10}$$

$$\text{HSI} : 8 * \text{ALT/AST} + \text{BMI}(+2, \text{if type 2 diabetes}; +2, \text{if female}).^{11}$$

$$\text{VAI} : [\text{WC}/39.68 + (1.88 * \text{BMI})] * (\text{triglycerides}/1.03) * (1.31/\text{HDL}), \text{for males}; [\text{WC}/36.58 + (1.89 * \text{BMI})] * (\text{triglycerides}/0.81) * (1.52/\text{HDL}), \text{for females}.^{18}$$

$$\text{TyG index} : \log[(\text{triglycerides})(\text{mg/dL}) \times \text{glucose (mg/dL)}/2].^{17}$$

Statistical analysis

In descriptive analyses, continuous variables were expressed as mean ± s.d. or as median with interquartile range [IQR], as appropriate and categorical variables as frequency and percentage. Numerical variables were compared using the Student's *t*-test for those that were normally distributed, and the Mann–Whitney *U*-test for those without normal distribution. The chi-square and Fisher's exact tests were used for qualitative data when appropriate.

Analysis of variance (ANOVA) with Bonferroni *post hoc* analysis and trend analysis was used to assess the differences in liver steatosis biomarkers values according to the histological steatosis grade. The correlation between the fatty liver biomarkers and the histological amount of liver steatosis was assessed with the Spearman's rank test. Pearson's coefficient was used to investigate the correlation between the steatosis biomarkers and insulin resistance as assessed with HOMA-IR. Receiver operating characteristic (ROC) curves were performed to investigate the accuracy of the five noninvasive biomarkers (FLI, NAFLD-LFS, HSI, VAI and TyG index) for estimating the presence of any steatosis (>5%), moderate/severe steatosis (>33%), NASH and advanced NASH using liver biopsy as the gold standard. The areas under the ROC (AUROCs) curves with 95% CI were recorded. Only for steatosis >5% and >33%, sensibility, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratio (LR) were calculated after the identification of the optimal cut-off point for each ROC curve using the Youden index. Multiple linear regression analyses were performed to evaluate the confounding effect of fibrosis and necroinflammation (ballooning and lobular inflammation) in the association between histologically assessed liver steatosis and its

Table 1 | Study group baseline characteristics (N = 324)

Age (years)	54 (45 60)
Males, *n* (%)	206 (64)
Alcohol consumption (g/day)	0 (0 5)
BMI (kg/m^2)	29 (26 33)
Waist circumference (cm)	101 (92 109)
Diabetes type 2, *n* (%)	133 (41)
Arterial HTN, *n* (%)	151 (47)
Hypertriglyceridemia, *n* (%)	181 (56)
Low HDLc, *n* (%)	97 (35)
Metabolic syndrome, *n* (%)	158 (50)
HOMA-IR	3.3 (2.3 5.6)
ALT (IU/L)	60 (41 89)
AST (IU/L)	40 (32 54)
GGT(IU/L)	65 (39 117)
US positive for steatosis, *n* (%)	241 (81)
Histological data	
Steatosis	40 (20 70)
None (<5%), *n* (%)	15 (5)
Mild (5 33%), *n* (%)	125 (39)
Moderate (33 66%), *n* (%)	97 (30)
Severe (>66%), *n* (%)	87 (27)
Advanced fibrosis (F3/4), *n* (%)	76 (24)
NASH, *n* (%)	171 (53)
Advanced NASH, *n* (%)	38 (12)
Steatosis markers	
FLI	80 (58 93)
NAFLD liver fat score	0.8 (0.5 2.4)
HSI	42.8 (38.6 47.8)
VAI	1.8 (1.2 3.2)
TyG	8.8 (8.4 9.3)

Data are expressed as median (IQR) and as frequency (percentage).

surrogate markers. Moreover, we used linear regression to evaluate the association between the steatosis biomarkers, histological grades of steatosis and insulin resistance as assessed with HOMA-IR.

Two-sided *P*-values of less than 0.05 were considered statistically significant. Statistical analysis was performed using Number Cruncher Statistical Systems 2008 (NCSS; Kaysville; UT, USA).

RESULTS

Study population and overall characteristics

Table 1 summarises the baseline features (clinical, laboratory and liver biopsy data) of the study population. The majority of patients were male (64%) and the mean age was 52.6 ± 11.43 years. Mean BMI was 29.7 ± 5.35 kg/m^2. Metabolic alterations were common and the full-blown metabolic syndrome was present in half of the cases. Of 296 patients, 241 (81%) had a liver ultrasound showing a bright liver pattern which was diagnostic of fatty liver. With regard to liver histology, only 5% of patients did not have steatosis, mild steatosis was detected in 39% and moderate/severe steatosis in the remaining 57% of cases. One hundred and seventy-one patients (53%) had NASH, 76 patients (24%) had advanced fibrosis (stage 3–4) and 38 (12%) had advanced NASH on liver biopsy.

Mean values of steatosis biomarkers were high: FLI 74 ± 23, NAFLD-LFS 1.3 ± 2.8, HSI 43.3 ± 6.9, VAI 3.0 ± 5.1, and TyG 8.9 ± 0.7.

Figure 1 shows the distribution of the five biomarkers according to the histological grades of steatosis. Except for VAI (ANOVA for trend, P = 0.065), the surrogate biomarkers of steatosis showed a linear trend across the histological grades of steatosis (ANOVA for trend, P < 0.001 for FLI, NAFLD-LFS, HSI and TyG index). However, their correlation with the histological amount of liver steatosis assessed as a continuous variable was only weak-to-moderate (Spearman's rho: 0.280 for FLI; 0.424 for NAFLD-LFS; 0.300 for HSI; 0.214 for VAI and 0.193 for TyG index).

Diagnostic accuracy of steatosis biomarkers for the detection of steatosis

The majority of indexes had significantly higher mean values in mild than in no steatosis grades (5–33% vs. 0–5%): FLI 70 ± 22 vs. 43 ± 25, respectively, P < 0.001; NAFLD-LFS 0.5 ± 2.7 vs. −0.9 ± 1.1, P = 0.299; HSI 42.3 ± 7.3 vs. 36.4 ± 4.5, P = 0.008; VAI 2.4 ± 2.2 vs. 0.8 ± 0.3, P = 1.000; and TyG 8.8 ± 0.6 vs. 8.0 ± 0.3, P < 0.001 (ANOVA with Bonferroni *post hoc* test), thus demonstrating the ability of these tests to confidently diagnose the presence of steatosis of any amount. However, only the FLI index and the NAFLD-LFS were able to discriminate between moderate and mild steatosis (33–66% vs. 5–33%): FLI 80 ± 20 vs. 70 ± 22, respectively, P = 0.004; NAFLD-LFS 1.9 ± 2.6 vs. 0.5 ± 2.7, P = 0.001; HSI 44.2 ± 6.2 vs. 42.3 ± 7.3, P = 0.185; VAI 3.7 ± 8.3 vs. 2.4 ± 2.2, P = 0.472; and TyG 9.0 ± 0.7 vs. 8.8 ± 0.6, P = 0.160 (ANOVA with Bonferroni *post hoc* test). None of these indexes distinguished between moderate and severe steatosis (33–66% vs. >66%): FLI 80 ± 20 vs. 77 ± 22, respectively; NAFLD-LFS 1.9 ± 2.6 vs. 2.2 ± 2.8; HSI 44.2 ± 6.2 vs. 45.1 ± 6.5; VAI 3.7 ± 8.3 vs. 3.3 ± 3.2; and TyG 9.0 ± 0.7 vs. 8.9 ± 0.7, all P = 1.000 (ANOVA with Bonferroni *post hoc* test) (Figure 1).

The diagnostic accuracy of the five surrogate biomarkers for steatosis >5% as detected by liver biopsy is

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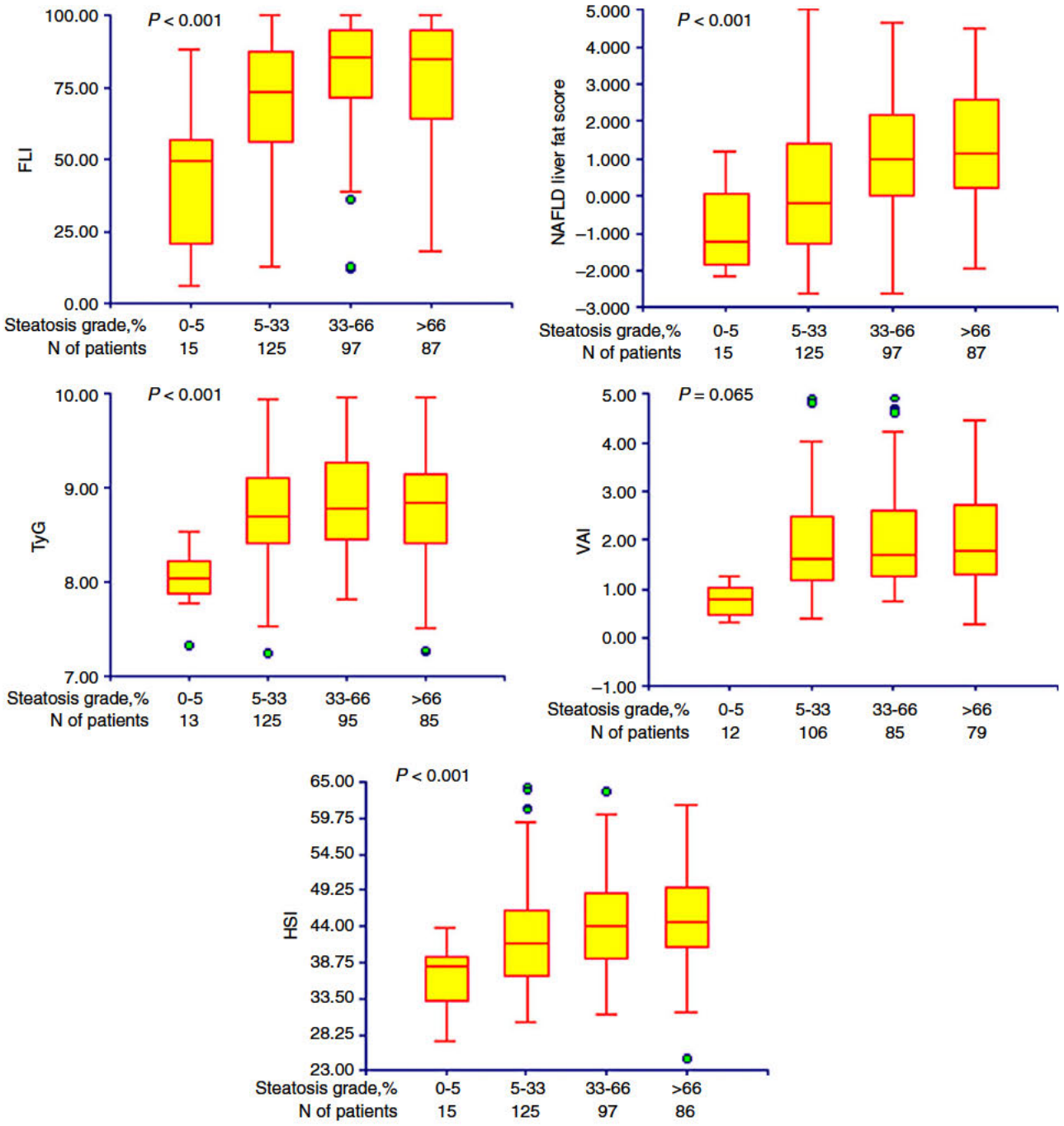

Figure 1 | Distribution of steatosis biomarkers according to the histological grade of steatosis. The yellow box represents the interquartile range. The red line across the box indicates the median. The 'whiskers' are the red lines that extend from the box to the highest and lowest values, excluding outliers (light green dots). ANOVA for trend analysis was performed to find out a linear trend across the steatosis grades.

shown in Figure 2. All the fatty liver markers displayed an acceptable accuracy in estimating the presence of steatosis of any amount vs. no steatosis. The VAI and TyG yielded the best AUROCs of 0.92 (95% CI 0.85–0.95) and 0.90 (0.84–0.94), respectively. FLI, NAFLD-LFS and HSI yielded AUROCs of 0.83 (0.72–0.91), 0.80 (0.69–0.88) and 0.81 (0.71–0.88), respectively. Table 2 shows the sensitivity, specificity, NPV, PPV and LR for estimating histological steatosis ≥5%, for the optimal cut-off of each steatosis biomarker, as determined by

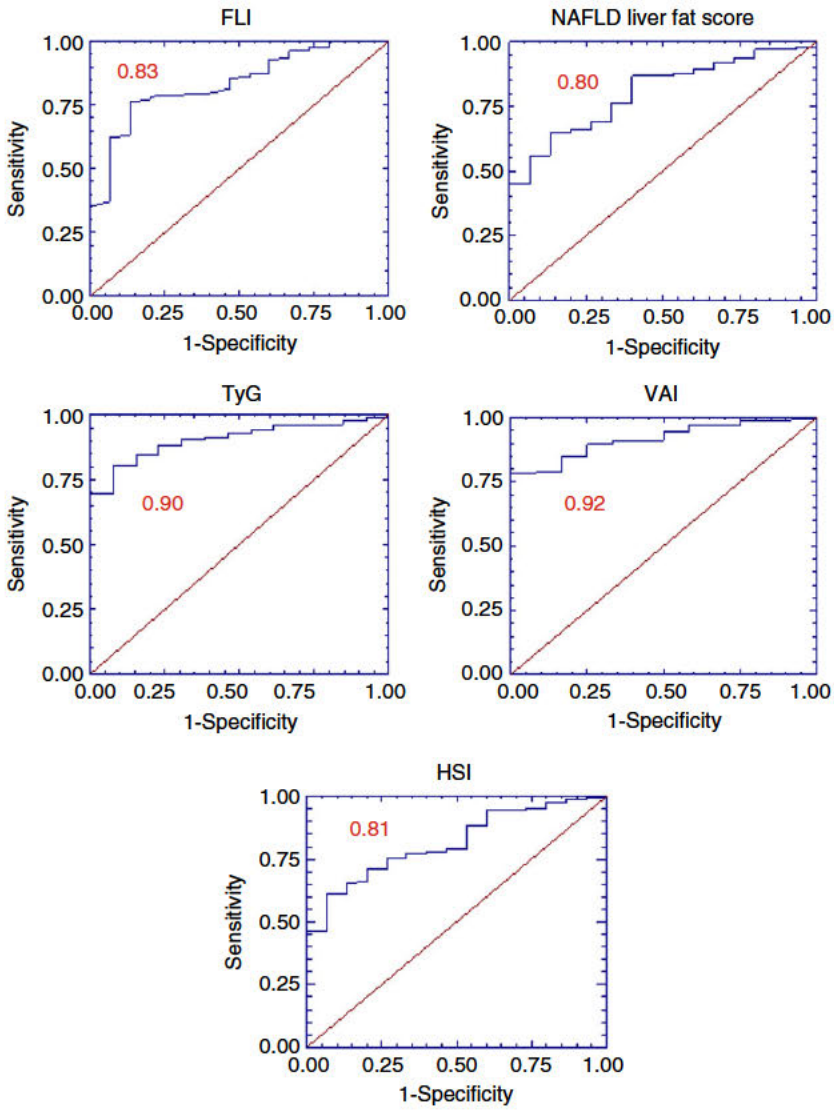

Figure 2 | AUROCs for diagnostic accuracy of steatosis biomarkers in comparison to liver biopsy (Any steatosis ≥5%).

applying the Youden method. FLI values greater than 60, NAFLD-LFS >0.16, HSI >41.6, VAI >1.25 and TyG >8.38, all had a PPV of 99% for predicting steatosis ≥5%.

The diagnostic performance of the five fatty liver biomarkers for estimating histological moderate/severe (>33%) vs. no/mild (0–33%) steatosis is shown in Figure 3 and Table 2. The accuracy of all these indexes in detecting the highest grades of steatosis (>33%) decreased to fair-very poor. NAFLD-LFS yielded the best AUROCs of 0.72 (0.66–0.77). With an optimal cut-off of >0.16 NAFLD-LFS predicted moderate/severe steatosis with sensitivity of 78% and specificity of 59%.

The diagnostic performance of the five steatosis biomarkers for the presence of steatosis diagnosed by ultrasound was rather poor: the AUROCs were 0.56 (0.48–0.64) for FLI, 0.71 (0.63–0.79) for NAFLD-LFS, 0.65 (0.58–0.73) for HSI, 0.60 (0.51–0.70) for VAI and 0.63 (0.54–0.71) for TyG. After restricting the analyses to the 241 patients with liver ultrasound indicative of hepatic steatosis [mean steatosis percentage 48 ± 22%; 156 (65%) with histological moderate/severe steatosis (>33%)], we confirmed the inability of all fatty liver markers to accurately predict more severe steatosis grades. Indeed, the AUROCs for estimating histological moderate/severe (>33%) vs. no/mild (0–33%) steatosis were 0.60 (0.53–0.68) for FLI, 0.69 (0.61–0.76) for NAFLD-LFS, 0.58 (0.50–0.66) for HSI, 0.57 (0.49–0.64) for VAI and 0.53 (0.46–0.61) for TyG.

Impact of fibrosis on steatosis biomarkers diagnostic accuracy

We next asked if fibrosis is a confounder of the relationship between steatosis biomarkers and the amount of liver fat. Patients with no or mild fibrosis (F0–F2) had a similar amount of histological steatosis as those with

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Table 2 | Diagnostic values of steatosis biomarkers for predicting steatosis in comparison to liver biopsy

	AUROC (95% CI)	Sensitivity,%	Specificity,%	PPV,%	NPV,%	LR
Steatosis >5%						
FLI (>60)	0.83 (0.72 0.91)	76	87	99	15	5.7
VAI (>1.25)	0.92 (0.85 0.95)	79	92	99	16	9.4
NAFLD liver fat score (>0.16)	0.80 (0.69 0.88)	65	87	99	11	4.9
TyG (>8.38)	0.90 (0.84 0.94)	80	92	99	16	10.4
HSI (>41.6)	0.81 (0.71 0.88)	61	93	99	10	9.2
Steatosis >33%						
FLI (>82)	0.65 (0.59 0.71)	59	69	71	56	1.9
VAI (>1.36)	0.59 (0.52 0.66)	74	41	64	53	1.25
NAFLD liver fat score (>0.16)	0.72 (0.66 0.77)	78	59	71	67	1.89
TyG (>8.75)	0.59 (0.52 0.65)	58	58	64	51	1.4
HSI (>43.0)	0.65 (0.58 0.70)	59	68	71	56	1.8

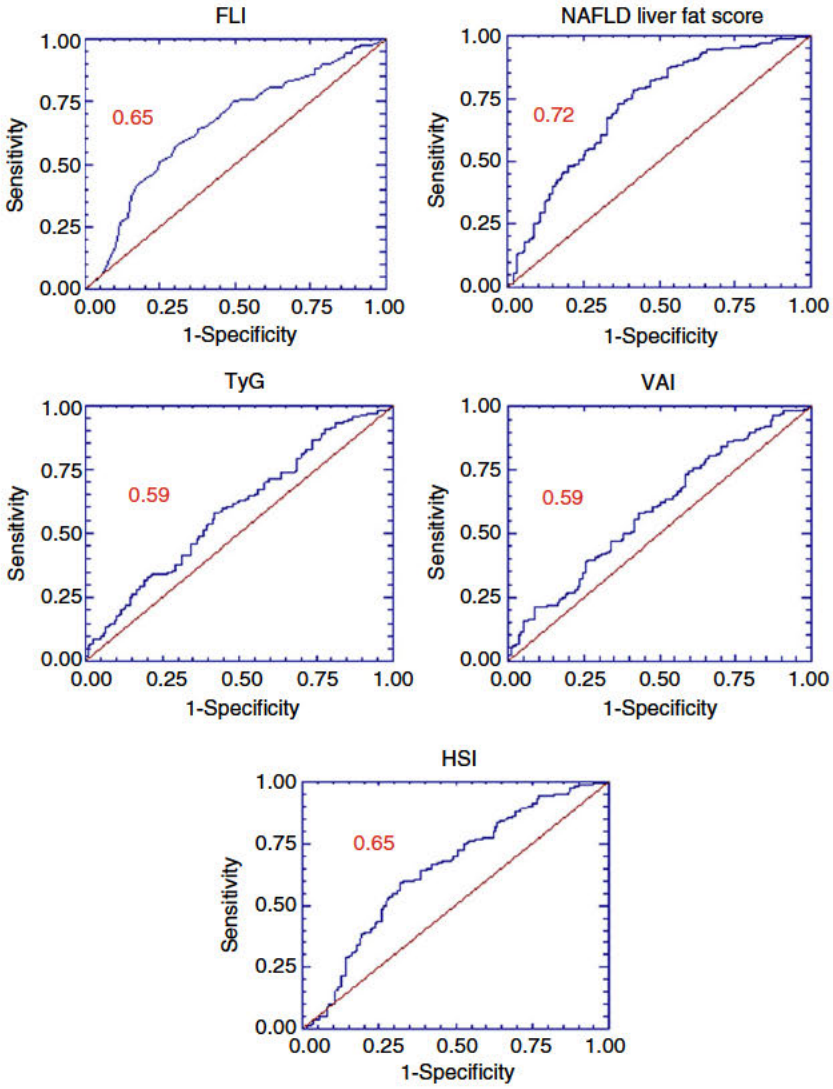

Figure 3 | AUROCs for diagnostic accuracy of steatosis biomarkers in comparison to liver biopsy (moderate/severe steatosis >33%).

advanced fibrosis (bridging fibrosis/cirrhosis, F3–F4): mean amount of steatosis: 41 ± 26% vs. 45 ± 21%, respectively, $P = 0.202$; steatosis >33% in 54% vs. 64% of cases, respectively, $P = 0.122$). Surprisingly, the mean values of all the surrogate biomarkers of steatosis were significantly higher in the presence of bridging fibrosis/

Table 3 | Mean ± s.d. values of steatosis biomarkers according to the amount of fibrosis

	No/Mild fibrosis (F0 2)	Advanced fibrosis (F3 4)	*P*
Histological steatosis	41 ± 26	45 ± 21	0.202
FLI	70 ± 23	84 ± 17	<0.001
VAI	2.8 ± 5.4	3.6 ± 3.7	0.233
NAFLD liver fat score	0.9 ± 2.5	2.6 ± 3.3	<0.001
TyG	8.8 ± 0.7	9.0 ± 0.7	0.013
HSI	43.1 ± 6.6	44.2 ± 7.9	0.223

cirrhosis (Table 3). This fibrosis-related confounding effect was more pronounced in the subgroup with no/mild steatosis (Figure 4). Multiple linear regression analysis confirmed that, except for HSI, all the surrogate biomarkers of steatosis were significantly correlated with fibrosis, independently from steatosis, as assessed with liver biopsy (Table 4).

Impact of necroinflammation on steatosis biomarkers diagnostic accuracy

In patients with NAFLD steatosis can exist either alone or in association with inflammation and liver cell injury.

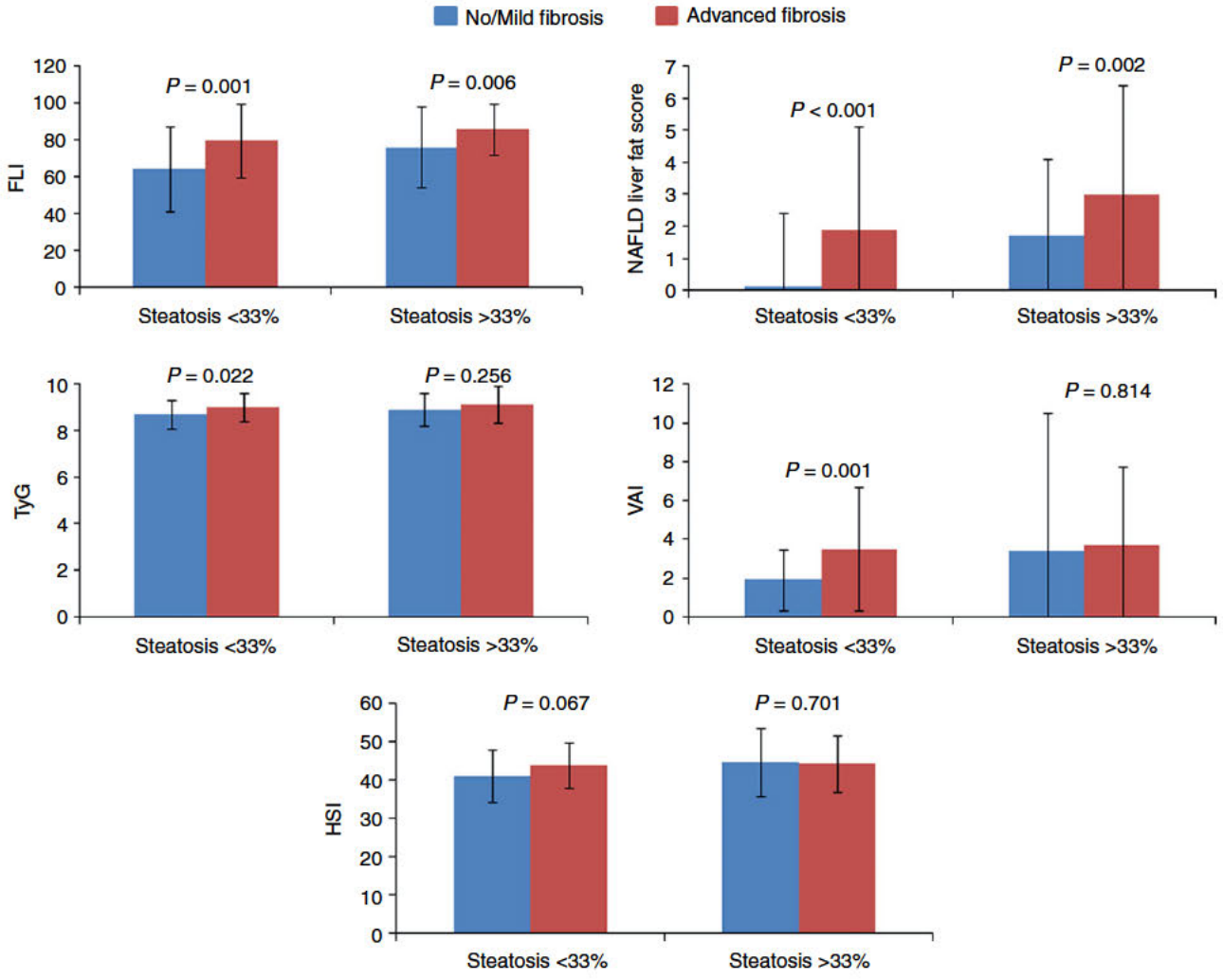

Figure 4 | Confounding impact of fibrosis on mean values of steatosis biomarkers. Data are represented as mean ± s.d. Student's *t*-test was performed for ascertaining differences between steatosis biomarkers according to fibrosis stage either for histologically assessed mild or moderate/severe steatosis.

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Table 4 | Multiple linear regression analysis assessing the impact of fibrosis, necroinflammation and insulin resistance on the relationship between steatosis biomarkers and histologically assessed liver steatosis

	FLI		VAI		NAFLD liver fat score		TyG		HSI	
	Beta coefficient	*P*	Beta coefficient	*P*	Beta coefficient	*P*	Beta coefficient	*P*	Beta coefficient	*P*
Histological steatosis (grades)	4.656	0.001	0.425	0.223	0.753	<0.001	0.099	0.023	1.722	<0.001
Histological fibrosis (Kleiner stages)	5.971	<0.001	0.552	0.030	0.623	<0.001	0.104	0.001	0.537	0.089
Histological steatosis (grades)	4.936	<0.001	0.450	0.203	0.680	<0.001	0.100	0.026	1.616	<0.001
Histological ballooning (grades)	2.892	0.132	1.494	0.003	0.397	0.082	0.131	0.032	0.461	0.438
Histological lobular inflammation (grades)	6.088	0.005	0.443	0.426	1.024	<0.001	0.051	0.461	1.197	0.075
Histological steatosis (grades)	5.995	<0.001	0.244	0.553	0.443	<0.001	0.072	0.119	1.537	0.001
HOMA-IR	1.066	<0.001	0.151	0.025	0.423	<0.001	0.040	<0.001	0.413	<0.001

Therefore, we investigated whether steatohepatitis is a confounder of the association between steatosis biomarkers and liver fat.

All five steatosis biomarkers were significantly higher in patients with NASH than in those without NASH. This was also true for all five markers in the subset of patients with no or mild steatosis (0–33%). In those with moderate/severe steatosis, FLI and the NAFLD-LFS were still significantly higher in patients with NASH than in those with steatosis alone (Table S1). Multiple linear regression analysis showed that FLI and the NAFLD-LFS were significantly correlated with lobular inflammation, and the VAI and the TyG scores with hepatocellular ballooning, independently from histologically assessed steatosis. Only HSI was not clearly associated neither with ballooning nor with inflammation (Table 4). Moreover, FLI, NAFLD-LFS and HSI were significantly associated with advanced NASH (Table S1). However, no panel of biomarkers predicted with acceptable accuracy NASH or advanced NASH [(AUROCs for NASH and advanced NASH, respectively: FLI 0.67 (0.61–0.73) and 0.69 (0.62–0.77); NAFLD-LFS 0.70 (0.64–0.76) and 0.75 (0.68–0.83); HSI 0.61 (0.55–0.67) and 0.61 (0.51–0.70); VAI 0.63 (0.56–0.69) and 0.59 (0.49–0.70); TyG 0.62 (0.56–0.68) and 0.57 (0.47–0.66)].

Correlation of steatosis biomarkers with insulin resistance as assessed with HOMA IR

Finally, given the close pathogenic link between steatosis and insulin resistance, we investigated if steatosis biomarkers were independently correlated with HOMA-IR.

As expected, the mean value of HOMA-IR in our cohort was increased (5.0 ± 5.4) and HOMA-IR showed a significant positive linear trend across the histological steatosis grades (steatosis <5%: 1.6 ± 0.8, 5–33%: 4.2 ± 5.9, 33–66%: 5.5 ± 5.4, >66%: 5.8 ± 5.1; ANOVA for trend: $P = 0.009$). All five steatosis biomarkers had a significant positive correlation with HOMA-IR. However, the strength of the correlation was weak-to-moderate for the majority of the surrogate biomarkers (Pearson's coefficient: 0.287 for FLI, 0.353 for HSI, 0.156 for VAI and 0.334 for TyG). NAFLD-LFS showed the strongest correlation with HOMA-IR (Pearson's coefficient: 0.859) which can be due to the fact that diabetes and insulin are enclosed in its formula. Multiple linear regression analysis confirmed that all the five steatosis biomarkers were significantly correlated with insulin resistance as

assessed with HOMA-IR independently from the histological grades of steatosis (Table 4).

DISCUSSION

This study attempted to independently validate in a head-to-head comparison and a large population of patients with liver biopsy for suspected NAFLD, five previously published panels of biomarkers of liver steatosis. Moreover, we inquired whether these markers can be used not merely for the detection but also for the quantification of steatosis. Importantly, we tried to determine whether fibrosis or steatohepatitis can confound their relationship with liver fat. We show that all five surrogate biomarkers of steatosis (FLI, NAFLD-LFS, HSI, VAI and TyG) are able to discriminate between the absence and the presence of steatosis with an overall good diagnostic performance. Moreover, all the biomarkers correlated with insulin resistance. Therefore, they should be useful screening tools to rule in NAFLD in patients with cardiometabolic risk factors. However, these biomarker panels are insufficiently accurate for the quantification of steatosis, and thus their value for monitoring changes in steatosis induced by pharmacological agents, diet and lifestyle measures or simply the natural course of the disease, will probably be limited. Unexpectedly, fibrosis and inflammation are important confounders of the relation between liver fat and these steatosis biomarkers. All markers were higher in patients with steatohepatitis than in those with steatosis alone even if the amount of liver fat was similar. Besides they all correlated with features of steatohepatitis (lobular inflammation, hepatocyte ballooning) independent of liver fat. Patients with advanced fibrosis had higher values for all these markers than those with minimal or no fibrosis despite adjustment for the amount of liver fat.

In the light of the hepatic and extrahepatic consequences of liver fat accumulation, there is a need for diagnosis and quantification of steatosis. Many studies have linked steatosis (defined by ultrasound) with cardiovascular outcomes including endothelial dysunction, early atherosclerotic lesions or coronary artery calcifications.[26–28] Steatosis can predict future cardiovascular events.[29] This prediction is often independent of traditional cardiovascular risk factors[30] therefore the identification of steatosis may be important for selecting patients at highest risk. Studies performed so far have not measured the risk in relation to the amount of liver fat as the diagnosis of steatosis was often made by liver ultrasound, an imaging method not suitable for quantification. Other studies have shown that steatosis predicts the occurrence of diabetes[31] and other components of the metabolic syndrome[32] including arterial hypertension.[33] Moreover, in type 2 diabetic patients treated with insulin, the amount of hepatic fat is the main determinant of daily insulin requirements.[34] Finally, the monitoring of patients treated for NASH, either by dietary or lifestyle interventions or future pharmacological therapies requires a reliable method of liver fat quantification. It has been shown that dietary interventions result in very early changes in steatosis that predict overall body weight and fat-mass reductions.[35] Therefore, a simple, inexpensive, readily available and, ideally, serum-based method of steatosis detection and quantification would be useful for the management of NAFLD patients.

Several surrogate biomarkers of steatosis have been proposed, but the methodology used for the initial validation and the study of their performances varies widely. Importantly, they have not been tested against liver biopsy, neither compared head-to-head in a single population. The Fatty Liver Index has been developed in a cohort of patients from the general population with ultrasound-diagnosed hepatic steatosis, either of alcoholic or metabolic origin.[9] The strength of FLI as a surrogate marker comes from the demonstration that it can predict clinical outcomes related to the metabolic syndrome. The FLI has been associated with hepatic, cardiovascular and cancer-related mortality, and also with reduced insulin sensitivity, risk of type 2 diabetes, accelerated atherosclerosis and cardiovascular risk.[12, 13] Other studies confirmed an association of FLI with all-cause and cardiovascular mortality in patients at high risk of coronary artery disease.[36] While clinically relevant, these associations might be conveyed independently of steatosis *per se* since the FLI calculation is build upon anthropometric or biological measures that are part of the phenotype of the metabolic syndrome. In fact, some studies have found only a limited correlation between FLI and the amount of liver fat *per se*, as defined by magnetic resonance spectroscopy[37, 38] and no other quantitative assessment of FLI vs. liver fat or changes in liver fat are available. Hence, the adequacy of FLI as a quantitative biomarker of steatosis is unknown. The NAFLD liver fat score has been developed against magnetic resonance spectroscopy as a measure of liver fat but the correlation of this marker with hepatic histology has not been studied.[10] Although there is limited independent validation[15] one study has shown that NAFLD-LFS can predict incident diabetes.[39] The Hepatic steatosis index has been developed from a large case–control study involving more than 5000 Korean patients with ultrasound-detected

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NAFLD.[11] External validation is so far limited and the correlation with liver histology has not been studied. However, the HSI seems to predict incident metabolic syndrome.[40] VAI is a marker of visceral adipose dysfunction (increased lipolysis, imbalanced adipocytokine production) associated with cardiovascular events and inversely correlated with insulin sensitivity.[16] Given the strong link between the expansion of an inflammed insulin resistant visceral adipose tissue and NASH[41] and the fact that at least two-thirds of the liver fatty acids are derived from circulating free fatty acids[42] it was anticipated that VAI would be associated with liver fat. The independent association between VAI and histologically defined steatosis has been demonstrated in genotype 1 infected HCV patients[18] but was not reported in patients with NAFLD.[21] Finally, the TyG index, which is the product of fasting triglycerides and glucose, inversely correlates with reduced insulin sensitivity determined by glucose clamp studies[17] and predicts the occurence of type 2 diabetes.[43] The TyG index independently predicted moderate-to-severe steatosis in patients with genotype 1 chronic HCV[19] but has not been studied in NAFLD.

This study reports for the first time on the association between two surrogate markers of insulin resistance, the VAI and the TyG index, and histologically defined hepatic steatosis in an homogenous population of NAFLD patients. This confirms the pathophysiological link existing between visceral adiposity, insulin resistance and hepatic steatosis. While both markers were performant at diagnosing the presence of steatosis, they were insufficiently discriminative between mild, moderate and severe steatosis and therefore unable to provide a reliable quantification of liver fat. The same holds true for FLI, NAFLD-LFS and HSI, initially validated as steatosis biomarkers. These data help better define the role these biomarkers may have in clinical practice. First, they can be useful for the diagnosis of hepatic steatosis in retrospective series where ultrasound is unavailable. Second, they can identify patients with steatosis among those exposed to cardiometabolic risk factors, all five biomarkers with similar diagnostic accuracy. Indeed, in most settings, hepatic ultrasound is not a routine examination in cardiovascular and metabolic screening. It is also an operator-dependent procedure, usually performed by a radiologist and with a low sensitivity especially for mild steatosis,[44] which limits its interest for large-scale, population-based screening. For the practicing hepatologist, the qualitative diagnosis of steatosis can be useful for the broad categorisation of patients as NAFLD or non-NAFLD. Indeed, from a liver perspective, quantification of steatosis is of low clinical relevance, given that the amount of liver fat per se does not predict liver disease progression. From a metabolic perspective; however, if the extrahepatic complications of steatosis turn out to be clinically relevant for the prediction of the complications of the metabolic syndrome (*vide supra*), then additional quantitative information may become important. Third, in their current form, none of these steatosis biomarkers can be used for segregating patients with low, moderate or high liver fat content, as this study clearly showed a plateau effect for all five biomarkers with increasing amounts of steatosis, defined histologically. Interestingly, a recent study in type-2 diabetic patients has shown that the performance of three surrogate markers, including FLI and HSI, for the prediction of steatosis, was very low. This finding from a population at high risk of moderate-to-severe steatosis suggests a limited ability of these surrogate biomarkers to quantitate steatosis, which is in agreement with the results presented here.[37] This lack of quantitative effect does not support the use of these markers for monitoring changes in liver fat in the setting of pharmacological or nonpharmacological (diet and lifestyle) interventions or even during the natural course of the disease. Moreover, more specific serum markers of liver fat need to be identified since this study has shown that hepatic inflammation and fibrosis significantly impact all these indices and may confound the relationship with liver fat. Of note, the interaction of the TyG index and the VAI with hepatic inflammation has already been shown by Petta *et al.* in patients with chronic hepatitis C[19] and the association between VAI and hepatic inflammation and fibrosis has been described in a smaller series of NAFLD patients[21] by the same author. This clearly denotes the lack of specificity of these markers for hepatic steatosis. Finally, we showed that all five surrogate biomarkers of steatosis were correlated with insulin resistance as assessed with HOMA-IR independently from the histological grades of steatosis. FLI and NAFLD-LFS have been correlated with insulin resistance and type 2 diabetes incidence in several studies.[12, 13, 39, 45] Interestingly a recent study among nondiabetic patients has demonstrated that NAFLD-LFS, HSI and FLI had modest accuracy in detecting steatosis but were independently associated with insulin sensitivity and secretion.[38] With regard to the other two markers, TyG was specifically developed to predict insulin resistance and was demonstrated to have better performance than HOMA-IR and to predict the development of diabetes.[17, 43, 46, 47] Finally, VAI has been associated with insulin resistance and diabetes incidence in different settings.[48–50] Our study confirms that, despite their limitations in assessing steatosis amount

but in parallel with the satisfactory performance in detecting steatosis, these surrogate biomarkers might be used as rough clinical estimates of insulin resistance, the major determinant of NAFLD.

This study has several strengths. It is a large series of patients with suspected NAFLD with available liver histology, the reference standard for the assessment of liver injury. This allows for the simultaneous assessment of the confounding effect of hepatic lesions other than steatosis, mainly necroinflammation and fibrosis. It is a head-to--head comparison of all five biomarkers thus avoiding pitfalls related to spectrum effect of the sampled population, at least as far as the direct comparison between the markers. There are also some limitations. It is a retrospective study in a selected population of patients referred to a tertiary care liver clinic. Although most patients were exposed to cardiometabolic risk factors, they might not be representative of the large population of similarly exposed patients without any indication of liver dysfunction. By studying the correlation between hepatic fat content determined by magnetic resonance spectroscopy and FLI and NAFLD-LFS, and reviewing discordant results,[37, 38, 51] others have emphasised the significant impact of the selection of the study population.[38] Future studies should incorporate comercially available serum markers such as Steatotest, which were not available for a majority of patients in this series. New imaging techniques such as the controlled attenuation parameter have been shown to have a good correlation with steatosis.[52, 53] They are advantageously coupled with the measurement of liver stiffness within the same procedure,[54] but therefore share some of the drawbacks of elastometry, especially in an overweight NAFLD population.[55] A thorough assessment of these promising techniques for their ability to truly quantitate steatosis and for their analytical variability[56] is warranted.

In conclusion, there is a strong medical need for clinical and research purposes of simple, noninvasive, cost-effective and reliable tools for diagnosing and quantifying hepatic steatosis. The panels of biomarkers so far available are performant for the detection of fatty liver and the prediction of insulin resistance, but lack the ability to quantitate liver fat and therefore are of limited clinical utility. Moreover, they are prone to substantial confounding due to steatohepatitis and advanced fibrosis. More research is needed to identify truly independent and quantitative markers of steatosis.

AUTHORSHIP

Guarantor of the article: Fabio Nascimbeni.

Author's contributions: Vlad Ratziu: study concept and design; Fabio Nascimbeni: analysis and interpretation of data; Larysa Fedchuk: acquisition, analysis and interpretation of data. All the authors contributed to drafting and critically revising the manuscript and approved the final document.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Mean ± s.d. values of steatosis biomarkers according to the presence of NASH and advanced NASH.

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Original Article

Prevalence of steatosis and insulin resistance in patients with chronic hepatitis B compared with chronic hepatitis C and non-alcoholic fatty liver disease

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ABSTRACT

The association of NAFLD with chronic hepatitis C (CHC) has been extensively studied but little is known about its coexistence with chronic hepatitis B (CHB).

Aims: To investigate the prevalence and determinants of steatosis and insulin resistance (IR) in CHB and its consequences on liver injury compared with CHC and NAFLD.

Methods: Patients with CHB (N = 110), CHC (N = 111) and NAFLD (N = 136) were evaluated by biomarkers of steatosis (SteatoTest > 0.38 as a surrogate for steatosis >5%), IR (HOMA IR > 2.7 as a surrogate for IR) and fibrosis (FibroTest > 0.48 as a surrogate for significant fibrosis, ≥F2).

Results: HOMA IR gradually increased in CHB, CHC and NAFLD: 2.3 ± 1.8; 3 ± 2.6 and 3.8 ± 2.7 ($p < 0.001$). The prevalence of steatosis >5% was 21% (CHB), 43% (CHC) and 82% (NAFLD), ($p < 0.001$). The prevalence of fibrosis ≥ F2 was 10% (CHB), 42% (CHC) and 21% (NAFLD), $p < 0.001$.

In CHB, IR was related to host and not viral factors. CHB patients with steatosis had higher BMI (29 ± 5.7 kg/m^2 vs. 24 ± 4 kg/m^2, $p < 0.001$), waist circumference (96 ± 14 cm vs. 84 ± 11 cm, $p = 0.001$) and HOMA IR (3.9 ± 2.6 vs. 1.8 ± 1.2, $p < 0.001$) than those without steatosis. HOMA IR independently predicted steatosis in CHB (OR = 1.9, 95% CI, 1.09 3.27, $p < 0.05$) and CHC (OR = 1.38; 95% CI, 1.07 1.78, $p < 0.02$). In CHB, metabolic risk factors and HOMA IR were not associated with significant fibrosis. HOMA IR was an independent predictor of fibrosis in CHC.

Conclusions: Steatosis may co exist in CHB patients but with a lower prevalence than in CHC and NAFLD. In CHB steatosis is related to host and not viral factors, and is not associated with the severity of fibrosis.

1. Introduction

Growing evidence suggests that non alcoholic fatty liver disease (NAFLD) is increasingly prevalent in the general population and be comes one of the leading causes of chronic liver disease [1]. The number of NAFLD patients at risk for disease progression or advanced disease is continuously growing and translates into increased overall and liver related mortality [2]. Insulin resistance and the metabolic syndrome are associated with disease progression in NAFLD [3] but this could also be true in patients with other causes of chronic liver disease.

Many studies focused on the prevalence and mechanisms of hepatic steatosis in patients with viral hepatitis. In chronic hepatitis C (CHC), steatosis can be related to both host metabolic risk factors, particularly in infections by genotypes 1 and 4 [4], and to the virus itself (genotype 3) [5]. The metabolic syndrome and insulin resistance are also responsi ble for fibrosis progression and poor response to interferon therapy [6, 7]. Conversely, in chronic hepatitis B, the prevalence and mechanisms associated with hepatic steatosis has not been clarified yet and only a few studies addressed this issue. The reported prevalence of hepatic steatosis in HBV infected patients varies widely (range from 14% to 70%), mainly because of heterogeneity of the patient population across studies [8]. Overall, hepatic steatosis affects almost a quarter of CHB

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; CHB, chronic hepatitis B; CHC, Chronic hepatitis C; HOMA-IR, homeostasis model assessment; HBsAg, hepatitis B surface antigen; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

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patients, a prevalence close to that in the general population and clearly lower than that in CHC [9]. Preliminary data from HBV patients also sug gests that insulin resistance and metabolic risk factors are associated with advanced disease and increased risk for hepatocellular carcinoma [10].

The aim of this study was to assess the interplay between the virus, the host and the liver lesions of steatosis and the advanced fibrosis in CHB as compared to CHC and NAFLD where these interconnections are well described. Specifically, we aimed to study the prevalence of steatosis and its more severe form, steatohepatitis (NASH), in HBV infected pa tients and to analyze the association with viral and host factors, particu larly metabolic syndrome and insulin resistance. We also investigated the effect of metabolic risk factors and insulin resistance on the risk of de veloping "superimposed" NASH and significant fibrosis.

2. Patients and methods

We prospectively included untreated patients with CHB and CHC eval uated in GHPS center by non invasive biomarkers for liver fibrosis and steatosis between July 2010 and April 2013. All CHB patients had positive hepatitis B surface antigen (HBsAg) and detectable serum HBV DNA (COBAS AmpliPrep/COBAS TaqMan Roche, v2.0; limit of detection 20 UI/ml). All CHC patients had antibodies against HCV and detectable HCV RNA. HCV genotyping and quantification of HCV RNA was performed for all patients (Abbott RealTime HCV M2000sp M2000rt, Abbott; limit of detection 12 UI/ml). A third group of patients with presumed NAFLD and therefore high likelihood of steatohepatitis and metabolic syndrome was considered the reference standard. Patients were considered to have NAFLD if they had evidence of steatosis on ultrasound or elevated aminotransferase levels (normal upper limit 35 IU/l) and one or more metabolic risk factors: overweight or obesity (BMI $\geq$ 25 kg/m^2), waist circumference 102 cm in male and >88 cm in female, hypertension (blood pressure > 130/85 mm Hg), type two diabetes or fasting plas ma glucose $\geq$ 6.1 mmol/l, triglyceride levels > 1.6 mmol/l and HDL cholesterol < 1.04 mmol/l for males and <1.29 mmol/l for women.

Patients were excluded if they had other causes of chronic liver disease (autoimmune hepatitis, hemochromatosis, Wilson disease, alpha 1 antitrypsin deficiency), mixed HBV/HCV or HIV co infection, alcohol consumption greater than 40 g/day. Other exclusion criteria were: decompensated cirrhosis (Child Pugh B and C), hepatocellular carcinoma and previous antiviral therapy. Finally 110 CHB, 111 CHC and 136 NAFLD patients met the inclusion and exclusion criteria and were considered for this study.

2.1. Clinical and laboratory assessment

Clinical and anthropometric data were collected on the day of the blood sampling: age, sex, ethnicity, weight, height, waist circumference, alcohol consumption, previous medical history and concomitant treat ment. Because of the heterogeneity of the study population and the high prevalence of non Caucasian among HBV infected patients, overweight and obesity were defined using ethnic specific criteria as appropriate [11]. Laboratory tests included: liver function tests, total cholesterol and triglyceride levels, blood creatinine level, serum ferritin, fasting blood glucose and insulin levels. Insulin resistance was determined by the homeostasis model assessment (HOMA IR) method using the following formula: HOMA IR = [fasting glucose (mmol/l) × fasting insulin (μUI/ml)] / 22.5. Insulin resistance was defined as HOMA IR > 2.7 according to previous publications for European countries [12].

2.2. Non invasive assessment of liver steatosis and fibrosis

FibroTest, SteatoTest, ActiTest and NashTest (BioPredictive, Paris, France) were determined for each patient as previously published [13]. The predetermined conversion value of FibroTest for METAVIR fibrosis stages was 0.00 0.27 for F0; 0.28 0.48 for F1; 0.48 0.58 for F2; 0.58 0.74 for F3; >0.74 for F4 [14 16]. The predetermined conversion of ActiTest for METAVIR activity grade scoring system was: 0.00 0.17 for A0; >0.17 0.52 for A1; >0.52 0.62 for A2; >0.62 for A3 [17]. The predetermined SteatoTest conversion for steatosis grade was: 0.00 0.38 for S0 (no steatosis); >0.38 0.57 for S1 (1 5%); >0.57 0.61 for S2 (>5% 32%); >0.61 0.69 for S2 (33 66%); >0.69 for S3S4 (>66%) [18]. NashTest was used for the prediction of NASH according to the pat ented algorithm in 3 classes as follows: 0.25 "no NASH"; 0.50 "possible NASH" and 0.75 presumed NASH [19]. The following definitions were used in this study: steatosis 1% 5% if SteatoTest > 0.30; (>5%) if

Table 1
General patients characteristic.

Characteristic	NAFLD (N = 136)	VHC (N = 111)	VHB (N = 110)
Age, years (mean ± sd)	57 ± 12	54 ± 11	42 ± 13*,§
Sex (n, %)	82 (60%)	50 (45%)*	64 (58%)
BMI kg/m^2 (mean, sd)	29.5 ± 5.2	25.8 ± 5.6*	24.9 ± 4.8*
Waist circumference, cm (mean, sd)	102 ± 13	90 ± 15*	86 ± 13*
Diabetes (n, %)	52 (38.5%)	13 (12%)*	8 (7.6%)*
HTA (n, %)	72 (53%)	32 (29%)*	19 (18%)*
Tobacco (n, %)	23 (17%)	29 (26%)	14 (13%)§
ASAT, IU/l (mean ± sd)	36 ± 16	52 ± 35*	34 ± 20§
ALAT, IU/l (mean ± sd)	46 ± 28	64 ± 52*	35 ± 37§
GGT, IU/l (mean ± sd)	69 ± 60	70 ± 77	31 ± 24*,§
Bilirubin (micromol/l)	10.6 ± 7.5	9.98 ± 7.2	9.85 ± 5.4
Fasting glucose, mmol/l (mean ± sd)	5.96 ± 1.64	5.27 ± 1.03*	5.23 ± 1.21*
Fasting Insulin (μIU/ml)	14.24 ± 8.6	12.5 ± 10.6	9.4 ± 6.3*,§
Cholesterol mmol/l (mean ± sd)	4.94 ± 1.25	4.85 ± 1.13	4.58 ± 1.17
Triglycerides mmol/l (mean ± sd)	1.59 ± 1.92	1.06 ± 0.95*	0.84 ± 0.40*,§
Liver injury presumed by blood tests			
No steatosis	13 (10%)	47 (42%)	71 (65%)
1–5% mild	11 (8%)	16 (14%)	16 (15%)
6–32% moderate	36 (27%)	32 (29%)	13 (12%)
33–66% marked	29 (21%)	8 (7%)	7 (6%)
>66% severe	47 (35%)	8 (7%)	3 (3%)
Presumed NASH	10 (7%)	5 (5%)	0 (0%)*
Possible NASH	84 (62%)	36 (32%)*,§	20 (18%)*,§
Necro-inflammatory activity (A2A3)	15 (11%)	25 (23%)*,§	3 (2.7%)*,§
Significant Fibrosis (F2F3F4)	29 (21%)	47 (42%)*,§	11 (10%)*,§

Significance level was set for $p < 0.05$.
* vs NAFLD.
§ VHB vs. VHC.

SteatoTest > 0.38; significant (≥F2) fibrosis if FibroTest ≥ 0.48; NASH if NashTest = 0.75.

Methodological aspects for the interpretation of non invasive biomarkers were applied [20].

2.3. Statistical analysis

Continuous variables were expressed as mean ± SD (standard deviation) and compared using Student *T* test or one way analysis of variance for multiple comparisons as appropriate. Categorical var iables were expressed as frequency and percentage and compared using the Chi Square Test. Bonferroni correction was used for multi ple comparisons. To identify independent variables associated with insulin resistance, steatosis, steatohepatitis and fibrosis as measured by the HOMA IR index, SteatoTest, NASH Test and FibroTest we used multiple logistic and linear regression analysis. To avoid co linearity, variables used in SteatoTest, NASH Test and FibroTest algorithm (BMI, fasting glucose, cholesterol and triglyceride levels) were not separately included in the multivariate model. Also, fasting glucose, insulin, type 2 diabetes and HOMA IR were not included in the same multivariate model. All analyses were performed using IBM SPPS 21 MacOS statistical software (Chicago IL) and GraphPad Prism Version 6.0 software.

3. Results

3.1. Patient characteristics

Patient characteristics (110 CHB, 111 CHC and 136 NAFLD) are listed in Table 1. There were striking differences in ethnicity since Caucasians were only 19% of the CHB patients, while 54% in CHC and 93% in NAFLD patients ($p < 0.001$). 73% of CHC patients were infected with genotypes 1 and 4. The median HBV viral load was 502 IU/ml (IQR, 25th 75th per centile 63 3596 IU/ml). Among HBV infected patients, 44% (n = 48) had low viral load (<20,000 UI/ml), normal transaminases level (<40 IU/l) and no fibrosis (FT < 0.27) and were considered inactive carriers. Alcohol consumption was low in the entire cohort and only 3 patients consumed more than 30 g/day, while 86% consumed ≤10 g/day. Compared with NAFLD and CHC, patients with CHB were younger (42 ± 13 years vs. 57 ± 12 and 54 ± 11 years, $p < 0.001$), had lower BMI (24.9 ± 4.8 kg/m^2 vs. 29.5 ± 5.2 kg/m^2 and 25.8 ± 5.6 kg/m^2, $p < 0.001$) and lower waist circumference (86 ± 13 cm vs. 102 ± 13 cm and 90 ± 15 cm, $p < 0.001$). As expected, the prev alence of diabetes and hypertension was significantly lower in patients with CHB and CHC than in those with NAFLD (7.6% and 12% vs. 38.5%, $p < 0.001$ and 18% and 29% vs. 53%, $p < 0.001$) but without significant differences between patients with viral hepatitis. Cholesterol levels were similar between groups, but triglyceride levels were significantly lower in patients with viral hepatitis ($p < 0.001$). HOMA IR, SteatoTest and FibroTest gradually increased across the three etiological groups (Fig. 1A, B and C). CHB patients had lower prevalence of significant fibrosis (10%) vs. 42% in CHC patients and 21% in NAFLD ($p < 0.001$). Cirrhosis (F4, Fibrotest > 0.74) was present in 2 (1.8%) of CHB, 9 (8.1%) CHC and 8 (6%) NAFLD patients.

3.2. Prevalence and factors associated with insulin resistance

The prevalence of insulin resistance (HOMA IR ≥ 2.7) in CHB patients was not significantly different as compared to CHC patients (28% vs. 37%, p = 0.16) but was significantly lower than that in NAFLD patients (58%), $p < 0.001$. Patient characteristics according to the presence of insulin resistance and etiology of chronic liver disease are presented in Table 2. CHB patients with insulin resistance had a higher BMI, the prevalence of type 2 diabetes, high blood pressure and steatosis. In CHB patients, BMI and triglyceride levels were predictors of insulin resistance in multivariate analysis (OR = 1.15; 95% CI, 1.03

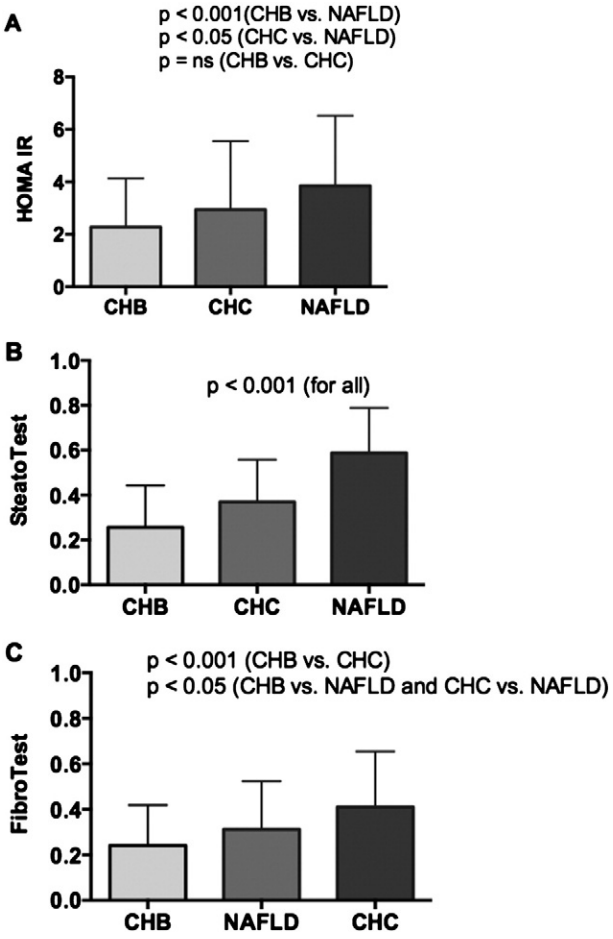

Fig. 1. A. HOMA-IR gradually increased across groups but the difference was not significant between CHB and CHC patients. B. SteatoTest significantly increased across groups with significant differences between pairs (CHB vs. CHC, CHB vs. NAFLD and CHC vs. NAFLD). C. FibroTest significantly increased across groups with significant difference between pairs (CHB vs. CHC, CHB vs. NAFLD and CHC vs. NAFLD).

1.29, $p < 0.02$ and OR = 3.63; 95% CI, 1.14 11.53, $p < 0.03$) independent of viral factors, similar to CHC (OR = 1.15; 95% CI, 1.04 1.27, p = 0.006 and OR = 2.18; 95% CI, 1.03 4.62, p = 0.04) and reference NAFLD patients (OR = 1.15; 95% CI, 1.05 1.25, p = 0.001 and OR = 2.06; 95% CI = 1.23 3.46, p = 0.006).

3.3. Prevalence and factors associated with steatosis and steatohepatitis

3.3.1. Steatosis

The prevalence of >5% steatosis gradually increased to 21% in CHB, 43% in CHC and 82% in NAFLD ($p < 0.001$), Table 1. Among patients with CHB, those with >5% steatosis had higher BMI (29 ± 5.7 kg/m^2 vs. 24 ± 4 kg/m^2, $p < 0.001$), waist circumference (96 ± 14 cm vs. 84 ± 11 cm, p = 0.001) and HOMA IR (3.9 ± 2.6 vs. 1.8 ± 1.2, $p < 0.001$) than those without steatosis despite similar age (44 ± 12 vs. 41 ± 13 years, p = 0.385), alcohol consumption (3 ± 7 vs. 5 ± 9 g/day, p = 0.27) and viral load (3.08 ± 1.5 vs. 3.03 ± 1.6 log, p = 0.9). In univariate analysis, metabolic risk factors (BMI, waist circumference, triglyceride levels) and insulin resistance (HOMA IR) were significantly associated with >5% steatosis regardless the etiology of chronic liver disease, Table 3. In multivariate analysis, high blood pressure (OR = 8.53; 95% CI, 1.05 68.8, $p < 0.05$) and waist circumference (OR = 1.14; 95% CI, 1.03 1.25, p = 0.006) were predictors of steatosis independent of viral load or Asian ethnicity. In HBV infected patients, HOMA IR and waist cir cumference were independent predictors of hepatic steatosis (OR = 1.9, 95% CI, 1.09 3.27, $p < 0.05$ and OR = 1.113, 95% CI, 1.006 1.231, $p < 0.05$)

Table 2
Patient characteristics according to the presence of insulin resistance and etiology of chronic liver disease.

	CHB (N = 110)			CHC (N = 111)			NAFLD (N = 136)		
	HOMA-IR > 2.7 N = 31	HOMA-IR < 2.7 N = 79	p Value	HOMA-IR > 2.7 (N = 41)	HOMA-IR < 2.7 (N = 70)	p Value	HOMA-IR > 2.7 (N = 79)	HOMA-IR < 2.7 (N = 57)	p Value
Age, years (mean ± sd)	42 ± 14	41 ± 13	0.74	53 ± 13	54 ± 10	0.8	58 ± 12	56 ± 12	0.3
Sex (%)	58	58	0.9	61	36	0.01	61	60	0.9
BMI kg/m^2 (mean, sd)	27.4 ± 5.7	23.9 ± 4.1	0.01	28.4 ± 5.3	24.3 ± 5.1	<0.001	31 ± 5	27.6 ± 5	<0.001
Waist circumference, cm (mean, sd)	92 ± 14	84 ± 12	0.01	99 ± 13	84 ± 14	<0.001	107 ± 13	95 ± 11	<0.001
Diabetes (%)	23	1.3	<0.001	20	7	0.06	47	27	0.02
High blood pressure (%)	33	12	0.009	42	21	0.03	56	50	0.5
Tobacco (n, %)	4 (13%)	10 (13%)	0.99	22	29	0.4	14	21	0.3
ASAT, IU/l (mean ± sd)	31 ± 7	35 ± 23	0.3	61 ± 44	47 ± 27	0.04	37 ± 16	36 ± 17	0.7
ALAT, IU/l (mean ± sd)	33 ± 12	36 ± 43	0.7	79 ± 63	55 ± 43	0.02	48 ± 30	44 ± 26	0.4
GGT, IU/l (mean ± sd)	37 ± 26	29 ± 23	0.1	77 ± 70	66 ± 80	0.4	69 ± 57	70 ± 65	0.8
Cholesterol mmol/l (mean ± sd)	4.40 ± 1.34	4.65 ± 1.10	0.3	4.65 ± 1.28	4.97 ± 1.02	0.15	4.77 ± 1.13	5.17 ± 1.38	0.07
Triglycerides mmol/l (mean ± sd)	0.96 ± 0.43	0.79 ± 0.39	0.06	1.34 ± 1.34	0.90 ± 0.56	<0.02	1.75 ± 0.93	1.37 ± 0.86	0.02
Presumed NASH	0	0	1	10	1	0.04	11	2	0.03
Possible NASH	32	13	0.02	63	21	<0.001	79	56	0.005
SteatoTest	0.37 ± 0.20	0.21 ± 0.16	0.01	0.48 ± 0.16	0.29 ± 0.16	<0.001	0.63 ± 0.18	0.52 ± 0.21	0.003
FibroTest	0.26 ± 0.20	0.23 ± 0.16	0.35	0.47 ± 0.28	0.37 ± 0.21	<0.04	0.33 ± 0.22	0.27 ± 0.19	0.12
Viral load (log)	2.69 ± 1.23	1.74 ± 0.20	0.21	5.7 ± 0.93	5.9 ± 0.67	0.3	–	–	

in a model including HBV DNA level and classical metabolic risk factors (systolic blood pressure, type 2 diabetes). When HBV inactive carriers were excluded from the analysis, HOMA IR was still a predictor of hepatic steatosis (OR = 1.73, 95% CI, 1.010 2.94, p < 0.05) independent of viral load.

3.3.2. Steatohepatitis

The prevalence of "possible" and "presumed" NASH in the entire cohort was 39.2% and 4.3% and gradually increased from 18.2%/0% in CHB to 32%/4.5% in CHC and 61.8%/7.4% in NAFLD patients respectively (p < 0.001).

The specificity of NashTest for the presumed NASH cutoff value (0.75%) is therefore 100% using CHB as control chronic liver disease and 94.5% using CHC, whatever the activity the necro inflammatory grade presumed by ActiTest.

The five patients with CHC and presumed NASH had similar character istics and metabolic profile than patients with NAFLD and presumed NASH: 4 had BMI ≥ 25, 4 insulin resistance, 3 elevated waist circumfer ence, 2 arterial hypertension, 1 diabetes type 2, 2 elevated fasting glucose, and 1 elevated triglycerides, Table 4.

3.4. Prevalence and factors associated with significant fibrosis

The prevalence of significant fibrosis (F2F4, FibroTest > 0.48) was 10%, 42% and 21% in patients with CHB, CHC and NAFLD. Factors associ ated with significant fibrosis in the entire cohort in univariate analysis were age, sex, etiology of chronic liver disease (p < 0.001), waist circum ference, high blood pressure and HOMA IR (p < 0.05). In multivariate analysis in the entire cohort, age, sex and the etiology of chronic liver disease were the strongest independent predictors of significant fibrosis (OR = 1.08, 95% CI, 1.05 1.10, p < 0.001; OR = 4.33, 95% CI, 2.22 8.45, p < 0.001; OR = 2.54, 95% CI, 1.75 3.69, p < 0.001). Factors associated with significant fibrosis in univariate analysis when patients were strat ified according to the etiology of chronic liver disease are presented in Table 5. In univariate analysis, waist circumference was associated with significant fibrosis both in CHB and NAFLD patients. In multivariate linear regression analysis adjusted for age and sex, waist circumference was in dependent predictor of fibrosis only in NAFLD (beta = 0.184, p = 0.02) but not in CHB patients (beta = −0.121, p = 0.16). HOMA IR was inde pendent predictor of significant fibrosis only in CHC patients, both in uni variate and multivariate analysis adjusted for age and sex (beta = 0.198, p < 0.02).

4. Discussion

This study specifically investigated the prevalence and determinants of insulin resistance, steatosis and steatohepatitis in CHB compared with CHC and NAFLD patients, the latter serving as a reference group with high prevalence of metabolic syndrome and insulin resistance.

The general thinking is that metabolic risk factors and insulin resis tance are highly prevalent in HCV but not in HBV chronic infection, sug gesting that HCV per se is a risk factor for insulin resistance [21]. In our study, the distribution of metabolic risk factors (i.e. type two diabetes, overweight and obesity) was similar between CHB and CHC patients but lower than in reference NAFLD group. Insulin resistance defined as HOMA IR ≥ 2.7, was present in almost one third of CHB patients.

The prevalence of hepatic steatosis in the literature in CHC and CHB varied according to the spectrum of steatosis and the cutoff used for its

Table 3
Factors associated with moderate to severe steatosis according to etiology of chronic liver disease in univariate analysis.

Variable	NAFLD N = 136		Chronic hepatitis C N = 111		Chronic hepatitis B N = 110	
	Correlation coefficient	p	Correlation coefficient	p	Beta	p
Age (yr)	0.023	0.8	0.073	0.45	0.159	0.09
Sex					0.140	0.14
BMI (kg/m^2)	0.390	<0.001	0.310	0.001	0.269	0.005
Waist circumference (cm)	0.321	<0.001	0.349	<0.001	0.329	0.001
HTA	0.074	0.4	0.135	0.15	0.354	<0.001
Diabetes	0.206	0.02	0.183	0.06	0.151	0.12
HOMA-IR	0.308	<0.001	0.443	<0.001	0.467	<0.001
Triglycerides (mmol/l)	0.298	<0.001	0.378	<0.001	0.208	0.04
Cholesterol (mmol/l)	0.099	0.25	0.086	0.4	0.30	0.75
Viral Load (log)	NA	NA	0.048	0.6	0.50	0.61
Viral Genotype	NA	NA	0.037	0.7	NA	NA

Table 4
Characteristics of patients with CHC and "superimposed" presumed NASH compared with patients with NAFLD and presumed NASH of the reference group.

	CHC patients with "superimposed" presumed NASH n = 5	NAFLD with presumed NASH n = 10	p
Age (years)	59 (46–72)	64 (55–73)	0.39
Sex (male, %)	1 (20%)	1 (10%)	1
BMI (kg/m^2)	28.4 (23.9–32.8)	33.4 (29.7–37.1)	0.09
Waist circumference (cm)	98 (80–116)	112 (102–122)	0.14
Type two diabetes (%)	1 (20%)	7 (70%)	0.12
High blood pressure (%)	2 (40%)	8 (80%)	0.25
ASAT (IU/l)	83 (3–163)	38 (27–50)	0.15
ALAT (IU/l)	80 (15–146)	54 (22–85)	0.18
GGT (IU/l)	106 (0–243)	70 (44–95)	0.58
Total cholesterol (mmol/l)	4.4 (2.9–5.8)	5.2 (4.5–6.0)	0.22
Triglycerides (mmol/l)	1.2 (0.7–1.6)	1.8 (1.2–2.5)	0.08
Fasting blood glucose (mmol/l)	6.0 (5.1–7.0)	7.4 (5.6–9.2)	0.30
Fasting insulin (μIU/l)	23 (0–49)	20 (12–29)	0.81
HOMA-IR	6.7 (0–15)	6.3 (3.7–9.0)	0.62
SteatoTest	0.62 (0.41–0.82)	0.69 (0.63–0.75)	0.50
ActiTest	0.63 (0.28–0.98)	0.30 (0.15–0.45)	0.04
FibroTest	0.70 (0.41–0.99)	0.41 (0.24–0.59)	0.04

binary definition [22]. This study confirmed that mild (1 5%) and moderate (5 32%) steatosis grades are the most prevalent both in CHC and for the first time in CHB (prevalence of mild 15% and moderate 12%). The present study confirmed the high prevalence (58%) of steatosis in CHC, in non genotype 3, mostly associated with metabolic factors. For the first time it was demonstrated that CHC patients with "presumed" NASH, have strikingly similar metabolic profile with NASH patients. Patients infected by HBV were mostly inactive carriers and in these patients none had a presumed NASH but 18% has possible NASH. ActiTest remained specific of necroinflammatory activity in CHB as in CHC without associated presumed NASH by NashTest.

The prevalence and the interplay between metabolic risk factors and HBV chronic infection is still a subject of debate and different studies have produced conflicting results. Unlike HCV infection, we confirmed that in CHB patients insulin resistance is mainly related to host but not viral factors. Similar to CHC and NAFLD, in CHB patients, BMI and triglyceride levels were independent predictors of insulin resistance. Interestingly, in our study, triglyceride levels were significantly lower in CHB vs. in CHC patients but unlike previous studies [22], no correlations were found between viral load and triglyceride levels. In CHB patients with steatosis had significantly higher BMI, waist circumference, HOMA IR and triglyceride levels but similar viral load as compared with CHB patients without steatosis.

Only 9% of CHB patients had more than 33% steatosis, which is close to the prevalence reported in the general population in Asian and Western surveys [11,23]. This prevalence is lower than reported by previous studies in HBV infected patients, but using more than 5% of steatosis the prevalence was 21%. Besides the binary definition variability, patients with excessive alcohol consumption have been excluded from our study and more than 80% of patients consumed less than 10 g/day.

Although the relationship between HBV virus and hepatic steatosis is still a subject of debate, most of the studies suggest a negative association between HBV DNA and liver fat [8]. Data from NAHANES III suggested that steatosis is probably not specifically related to HBV, as HBV carrier did not have higher prevalence of diabetes and obesity or higher levels of insulin resistance than the general population [24]. Thus, our results and those of previous studies, suggest that in CHB patients, steatosis is probably due to chance association with metabolic abnormalities predisposing to NAFLD rather than viral factors.

Some experimental data suggest that HBV encoded proteins can act as promoters of steatosis despite the above mentioned clinical data showing no such association. The HBx protein is thought to play a role in the development of hepatic steatosis via transcriptional activation of SREBP1 c and PPARγ that increase hepatic triglyceride production and lipid uptake [25]. This hypothesis was not confirmed by our results and those of most clinical studies that showed that host metabolic risk factors [26 29], are the main determinants of hepatic steatosis in CHB patients. Viral genotype, viral load or HBeAg status did not impact on liver fat content in a Chinese magnetic resonance spectroscopy study, further reinforcing the lack of association between HBV and liver steatosis [30].

Metabolic risk factors were not independent predictors of the severity of liver fibrosis in CHB patients. However these results should be interpreted with caution, as in our cohort the prevalence of advanced fibrosis (FT > 0.48, F2) was low among HBV infected patients. This could probably be explained by the relatively high prevalence of inactive carriers among HBV patients in our cohort. Insulin resistance was an independent predictor of fibrosis severity in CHC but not CHB patients. Only waist circumference was associated with fibrosis severity in CHB patients in univariate analysis, but this effect disappeared in multivariate analysis after adjusting for age and sex. This could be partially explained by the low prevalence of significant fibrosis in our CHB cohort.

The main limitation of our study is the lack of liver biopsy to assess the severity of histological lesions. However, we used robust non invasive biomarkers with extensive validation for the estimation of fibrosis stages, steatosis and activity grades or the presence of steatohepatitis in patients with chronic liver disease [18,19,31,32]. These non invasive

Table 5
Factors associated with significant fibrosis (≥F2) according to the etiology of chronic liver disease.

	CHB (N = 110)		CHC (N = 111)		NAFLD (N = 136)	
	Correlation coefficient	p Value	Correlation coefficient	p Value	Correlation coefficient	p Value
Age (years)	0.234	<0.02	0.384	<0.001	0.245	0.005
Sex	0.283	0.003	0.250	0.008	0.239	0.005
BMI (kg/m^2)	0.072	0.5	0.022	0.8	0.169	<0.05
Waist circumference (cm)	0.005	0.9	0.128	0.2	0.206	<0.02
Type two diabetes	0.040	0.6	0.146	0.12	0.105	0.22
High blood pressure	0.034	0.7	0.139	0.14	0.128	0.14
ASAT (IU/l)	0.008	0.9	0.406	<0.001	0.177	< 0.04
ALAT (IU/l)	0.043	0.6	0.279	0.003	0.036	0.67
GGT (IU/l)	0.234	<0.02	0.276	0.003	0.053	0.5
Total cholesterol (mmol/l)	0.188	0.05	0.281	0.003	0.275	0.001
Triglycerides (mmol/l)	0.021	0.8	0.058	0.5	0.046	0.59
Fasting blood glucose (mmol/l)	0.131	0.17	0.192	<0.05	0.126	0.14
Fasting insulin (μIU/l)	0.111	0.24	0.146	0.12	0.090	0.29
HOMA-IR	0.080	0.40	0.190	<0.05	0.131	0.12
HBV DNA (log)	0.058	0.5	–	–	–	–
HCV RNA (log)	–	–	0.038	0.7	–	–

markers were used by independent investigators in previous studies where biopsies were not available [19,33,34]. Another limitation of this study is the rather low number of patients with CHB that could have missed an association between IR and fibrosis in this subgroup.

5. Conclusions

In conclusion, we demonstrated that insulin resistance, metabolic risk factors and steatosis might coexist with HBV chronic infection but with a lower prevalence than in NAFLD patients. Contrary to HCV chronic infection, in HBV infected patients both insulin resistance and steatosis are related to host metabolic risk factors and not to virus per se and are not associated with fibrosis severity. Longitudinal long term follow up studies including patients with the whole spectrum of disease severity are needed to further understand the role of insulin resistance and met abolic risk factors in fibrosis progression in CHB patients.

Competing interests

Raluca Pais, Elena Rusu, Diana Zilisteanu, Alexandra Circiumaru, Laurentiu Micu and Mihai Voiculescu and Vlad Ratziu: there are no competing interests related to this work.

Thierry Poynard is the inventor of FibroTest/SteatoTest and the founder of BioPredictive, the company that markets these tests. Patents belong to the French Public Organization Assistance Publique Hôpitaux de Paris.

Authors' contributions

RP, TP performed the statistical analysis and drafted the manuscript and participated in study design. ER and MV participated in data collec tion and overviewed the manuscript. DZ, AC and LM participated in data collection and patients' enrolment. VR conceived of the study, par ticipated in its design and coordination and overviewed the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors state that they have no conflicts of interest.

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Article 4. The impact of obesity and metabolic syndrome on chronic hepatitis B and drug-induced liver disease. Clin Liver Dis. 2014 Feb;18(1):165-78

The Impact of Obesity and Metabolic Syndrome on Chronic Hepatitis B and Drug-Induced Liver Disease

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KEYWORDS

• Fatty liver • Steatosis • Metabolic syndrome • Chronic hepatitis B
• Drug-induced liver injury • Fibrosis • Cirrhosis • Hepatocellular carcinoma

KEY POINTS

- In chronic hepatitis B, steatosis is not more frequent than in the general population, a notable difference with the high prevalence seen in chronic hepatitis C.
- Both steatosis and insulin resistance (IR) are related to host metabolic risk factors and therefore occur mainly through epidemiologic association; viral factors (viral genotype, viral load, and hepatitis B e antigen status) do affect liver fat content or IR levels.
- Experimental data suggest that at the molecular level, the HB*x* protein might induce liver fat accumulation, but human studies do not show evidence for a viral-induced mechanism.
- A wealth of experimental data suggest that the steatotic liver is more susceptible to liver injury and that intrinsic abnormalities associated with excessive hepatic fat deposition in animal models should promote hepatotoxicity in patients with nonalcoholic or metabolic fatty liver disease.
- Diabetes and possibly obesity could favor methotrexate-induced liver fibrosis in patients treated for psoriasis.

CHRONIC HEPATITIS B

Because overweight and diabetes, the 2 main predisposing conditions for nonalcoholic or metabolic fatty liver disease (NAFLD), are increasingly prevalent worldwide,

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chronic hepatitis B (CHB) can frequently coexist with NAFLD. Type 2 diabetes and obesity are risk factors for fibrosis progression, cirrhosis, and related complications in the general population,[1,2] in patients with chronic hepatitis C,[3] and in patients with alcoholic liver disease.[4,5] In the following sections, the evidence linking these conditions to the severity of liver disease in patients with CHB is reviewed.

PREVALENCE AND DETERMINANTS OF STEATOSIS IN PATIENTS WITH CHB

The prevalence of steatosis in CHB ranges widely from 14% to 71%, with a median of 31% (**Fig. 1**, **Table 1**). This prevalence is close to that in the general population, as assessed by ultrasonography in Asian and Western surveys (20%–30%).[6,7] The prevalence is higher in European series[8–14] (46%) than in those from the Asia-Pacific region[15–24] (24%), which might be explained by differences in alcohol consumption (higher in some European studies) or in body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters). When only studies including patients without alcohol consumption are considered, the prevalence decreases to 26%.[25] Although most patients have mild steatosis, only a few (22%), have marked steatosis (ie, >30% of hepatocytes showing fat droplets). In chronic hepatitis C, the prevalence of steatosis (any degree of fat infiltration) was 69%, ranging from 62% to 76%.[25] Thus, the prevalence of steatosis in CHB (1) seems to be similar to that in the general population and (2) is most probably around 2-fold lower than in chronic hepatitis C virus (HCV) infection.

Steatosis in patients with CHB virus (HBV) infection is correlated exclusively with metabolic risk factors (BMI, diabetes, triglyceride levels) and no direct association has been identified with viral factors.[25] The same holds true for steatohepatitis when strictly defined, histologically.[26] This finding suggests that steatosis is caused by chance association with metabolic abnormalities predisposing to NAFLD rather than a direct role of the virus, as is the case with genotype 3 HCV. Data from the National Health and Nutrition Examination Survey III[27] have confirmed that HBV carriers do not have a higher level of insulin resistance (IR) (measured by Homeostatic Model Assessment levels), or higher prevalence of diabetes or obesity than the general population, and therefore steatosis is probably not specifically related to the presence of HBV. This finding was supported by Chinese magnetic resonance spectroscopy studies showing that HBV carriers younger than 60 years had a lower content of liver fat compared with non-HBV carriers, even after adjustment for alcohol consumption, demographic and metabolic factors, or dietary intake.[28] Further reinforcing the lack of an association between HBV and steatosis is the evidence that viral genotype, viral load or hepatitis B e antigen (HBeAg) status do not affect hepatic fat in the same Chinese series.[5,28]

Concerning the precise subtype of NAFLD, only 1 study with detailed histology is available. Of 163 US-based patients with HBV, half of whom were overweight or obese, the proportion of NAFLD was 19%.[26] On rigorous histologic criteria, two-thirds of these patients had steatohepatitis (13% of the total cohort).[26] This is a lower prevalence than would be expected from a non–HBV-infected population with the same level of increased body weight. Why HBV carriers would have a lower than expected prevalence of metabolic syndrome is unclear. It has been hypothesized that HBV might interfere with lipid metabolism and result in lower triglyceride and cholesterol levels.[28] It is also unknown whether the current, lower levels of metabolic syndrome, overweight, and IR in HBV carriers, especially in Asia, will still hold true with the future expansion of the prevalence of obesity and diabetes worldwide. Data from Wong and colleagues[20] show that the prevalence of metabolic syndrome clearly increases with age: patients with HBV older than 60 years have a 3-fold higher

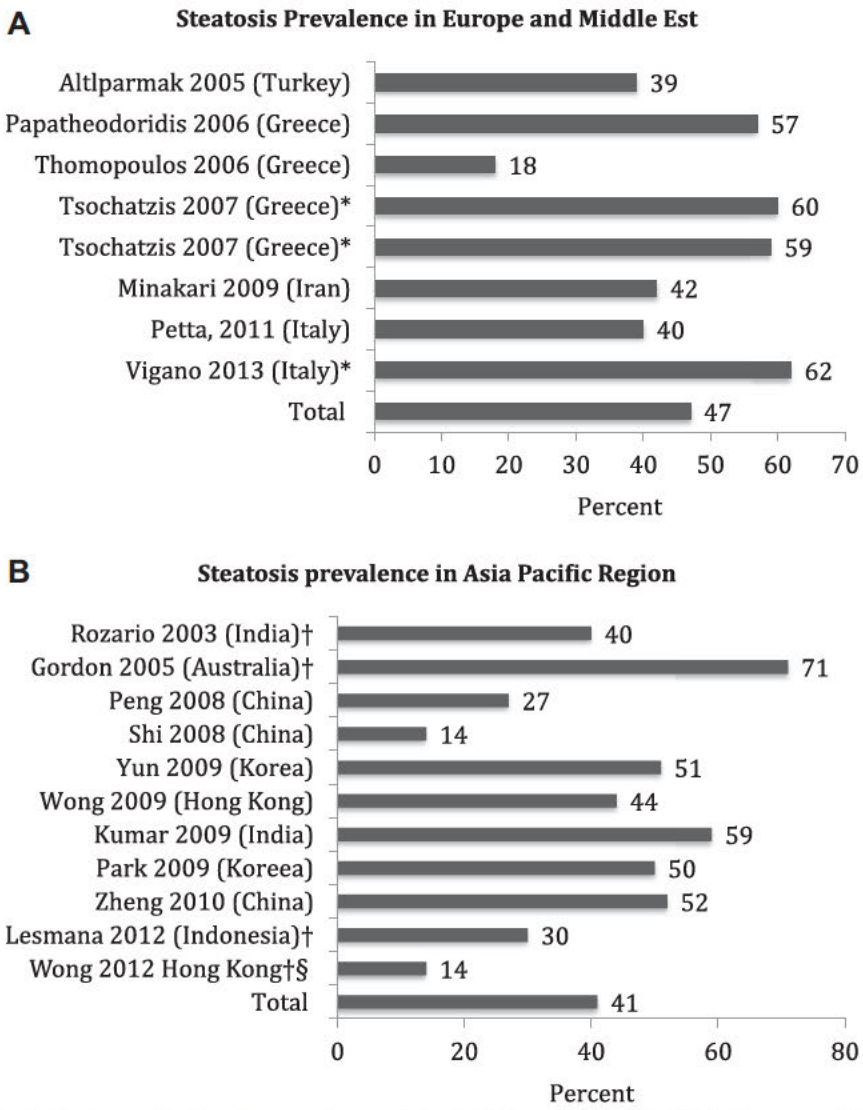

Fig. 1. Studies evaluating the prevalence of steatosis in patients with CHB in Europe and the Middle East (*A*) and Asia Pacific region (*B*). *Patients consuming alcohol not excluded; †patients drinking alcohol not excluded; §general population. (*Data from* Refs.[8–19,21–24,28,33,35])

prevalence of metabolic syndrome than those aged 21 to 40 years. It is therefore highly probable that in both the West and the East, the trends of obesity epidemics will considerably affect the association between steatosis and HBV infection.

MOLECULAR MECHANISMS AND FACTORS ASSOCIATED WITH STEATOSIS IN PATIENTS WITH CHB

In stark contrast with clinical data, there is some experimental evidence that HBV can induce hepatic steatosis, mainly through the HBV *X* (HBx) protein. Both the livers of

Table 1
Prevalence and distribution of hepatic steatosis in patients with CHB and chronic hepatitis C (CHC)

	Patients with CHB			Patients with CHC	
Reference	Patients (N)	Steatosis >5% (n, %)	Marked Steatosis >30% (n, %)	Patients (N)	Steatosis (n, %)
Europe and the Middle East					
Altlparmak et al,[9] 2005	164	64 (39)	19 (12)		
Papatheodoridis et al,[8] 2006	174	99 (57)	38 (22)	260	180 (69)
Thomopoulos et al,[10] 2006	233	42 (18)	11 (5)		
Tsochatzis et al,[11] 2007[a]	213	127 (59)	46 (22)	163	117 (72)
Tsochatzis et al,[12] 2007[a]	95	57 (60)	13 (14)	176	117 (66)
Minakari et al,[13] 2009	132	56 (42)	20 (15)		
Petta et al,[14] 2011	170	68 (40)	NR	170	80 (47)
Vigano et al,[35] 2013[a]	235	146 (62)	24 (10)		
Total	1416	659 (46)	171 (12)	769	494 (64)
Asia Pacific Region					
Rozario & Ramakrishna,[15] 2003[a]	82	33 (40)	NR	45	28 (62)
Gordon et al,[16] 2005[a]	17	12 (71)	3 (18)	74	53 (72)
Peng et al,[17] 2008	153	41 (27)	10 (6)		
Shi et al,[18] 2008	1915	260 (14)	25 (1)		
Yun et al,[19] 2009	86	44 (51)	2 (2)		
Wong et al,[33] 2009	266	116 (44)	21 (8)		
Kumar et al,[21] 2009	69	41 (59)	7 (10)		
Park et al,[22] 2009	80	40 (50)	NR		
Zheng et al,[23] 2010	204	106 (52)	83 (40)		
Lesmana et al,[24] 2012[a]	174	52 (30)	14 (8)		
Wong et al,[28] 2012[a,b]	91	13 (14)	NR		
Total	3137	758 (24)	165 (5)	119	81 (68)
Overall total	4553	1417 (31)	336 (7)	888	575 (65)

Abbreviation: NR, not reported.
[a] Patients drinking alcohol not excluded.
[b] General population.
Data from Refs.[8–19,21–24,28,33,35]

transgenic mice expressing the HB*x* protein and hepatoma cell lines transfected with the HB*x* protein accumulate excessive amounts of cellular fat.[29] In these experimental models, the HB*x* protein induces expression of SREBP1c and of PPAR-γ, an action involving activation of the PI3K/AKT pathway and downregulation of PTEN expression.[29] HB*x* might also interact with the liver X receptor to induce SREBP1c activation.[30] This interaction results in increased expression of downstream genes involved in lipogenesis and accounting for fat accumulation.[29,31] The relevance of these findings to hepatitis B infection in humans is questionable, because no data point toward a virus-induced steatosis in HBV carriers.

IMPACT OF STEATOSIS AND METABOLIC COFACTORS ON THE SEVERITY OF LIVER INJURY AND DISEASE OUTCOMES

Diabetes and obesity are recognized risk factors for fibrosis progression and development of cirrhosis in the general population[1,2] and in patients with chronic liver diseases, such as chronic hepatitis C (CHC), NAFLD, and excessive alcohol consumption.[4,5,32] In the following sections, available data on the respective impact of steatosis, clinical metabolic risk factors, and IR on liver-related outcomes in HBV-infected patients are reviewed.

Role of Steatosis

In chronic HBV infection, steatosis is not correlated with either necroinflammatory activity or fibrosis stage.[9,10,13,17,18,23,33] Thus, metabolic steatosis by itself is not a predictor of histologic severity; whether the same holds true for steatohepatitis is unknown. The only study that specifically looked at superimposed nonalcoholic steatohepatitis in patients with HBV did not investigate associations with histologic severity.[26] However, there are some data suggesting that steatosis might be associated with reduced HBV viral load.[9,17,18,23] This theory is difficult to reconcile with experimental studies (see later discussion) showing that HBV might promote lipogenesis. Nonetheless, 1 study in HBV transgenic mice fed either a high-fat diet or a control diet has shown that animals fed the high-fat diet developed full-blown histologic lesions of steatohepatitis, despite a reduction in HBV DNA and hepatitis B surface antigen (HBsAg) levels.[34] Other clinical studies did not confirm this reduction of viral replication in patients with HBV with liver steatosis.[28]

Role of IR and Metabolic Factors

Large studies in Asian patients with HBV have confirmed that the metabolic syndrome is associated with higher fibrosis stages and a higher prevalence of cirrhosis.[20] This association is independent of other known fibrosis cofactors and has been confirmed when cirrhosis was diagnosed both by histology and by elastometry. The probability of cirrhosis increased with increasing number of components of the metabolic syndrome, with diabetes mellitus and hypertension showing a strong association.[20] BMI was an independent predictor of advanced fibrosis in European-based patients with HBV.[35] Other studies confirmed that overweight[36] or diabetes[27] are associated with an increased risk of cirrhosis and liver-related death in male HBsAg carriers.[36] Neither of these studies had data on insulin, and therefore IR, as a confounder, could not be assessed. A Taiwanese longitudinal study (a substudy of the REVEAL cohort) of 1142 patients with HBV with a median of 7.8 years of follow-up has shown that a high serum insulin at baseline is a strong and independent predictor of cirrhosis occurrence (detected by ultrasonography) at follow-up.[37] The risk for cirrhosis related to a BMI greater than 25 kg/m^2 or to diabetes mellitus was greatly attenuated and became statistically nonsignificant after adjustment for insulin.[37] Studies evaluating the factors associated with fibrosis severity are summarized in **Table 2**.

Obesity and diabetes are independent risk factors for hepatocellular carcinoma (HCC) in the general population.[38–40] There are numerous studies showing that overweight/obesity or diabetes significantly increase the risk of HCC in HBV carriers. In a Taiwanese community cohort of 23,567 patients followed prospectively for 14 years,[41] the association of obesity and diabetes increased at least 10-fold the risk of HCC in HBV carriers (relative risk of 21 in nonobese, nondiabetic HBV carriers vs 265 in obese, diabetic HBV carriers, after adjustment for common carcinogenic confounders). Diabetes alone significantly increased the risk of HCC in HBV carriers (2.4-fold).[41] A

Table 2
Factors associated with fibrosis severity

Reference	Patients (N)	Prevalence of Steatosis (N, %)	Prevalence of Advanced Fibrosis (N, %)	Factors Associated With Advanced Fibrosis in Multivariate Analysis
Papatheodoridis et al,[8] 2006	174	99 (57)	46 (26)[c]	Diabetes[a]
Thomopoulos et al,[10] 2006	233	42 (18)	37 (16)[c]	Necroinflammation
Peng et al,[17] 2008	153	41 (27)	30 (20)	Necroinflammation
Shi et al,[18] 2008	1915	260 (14)	NR	Age, necroinflammation
Yun et al,[19] 2009	86	44 (51)	16 (19)	Necroinflammation
Persico,[83] 2009	126	14 (13)	NR	Age
Wong et al,[33] 2009	266	116 (44)	68 (26)[c]	Male sex, BMI, metabolic syndrome
Park et al,[22] 2009	80	40 (50)	45 (56)	HBeAg[b], portal/periportal inflammation
Zheng et al,[23] 2010	204	106 (52)	70 (34)	BMI, cholesterol
Petta et al,[14] 2011	170	68 (40)	44 (26)	Age, steatosis, necroinflammation
Vigano et al,[35] 2013	235	146 (62)	94 (40)	Age, HBeAg status, BMI, alcohol

[a] If patients with cirrhosis were excluded, no associations were found between type 2 diabetes and fibrosis stages.
[b] Negative association between HBeAg status and fibrosis severity.
[c] Cirrhosis.
Data from Refs.[8,10,14,17–19,22,23,33,35,83]

smaller but prospective Taiwanese study with long follow-up[37] reported that type 2 diabetes is a determinant of HCC independent of hepatitis viral infection thus confirming the universal carcinogenic potential of diabetes on the liver (ie, irrespective of the level of endemicity for viral hepatitis). Again, as is the case for fibrosis, IR seems to hold the key to the deleterious effect of diabetes or obesity. These investigators[37] showed that a high fasting insulin level on admission is an independent predictor of incident HCC in male HBV carriers. The association between overweight and HCC disappeared after adjustment for insulin, thus suggesting a more direct role for hyperinsulinemia than for obesity or diabetes. Also, this association between insulin and incident HCC increased in strength with longer exposure (>8 years of follow-up),[37] which strengthens the biological plausibility of this statistical association. There are intrinsic methodological limits to the possibility of detecting an association between diabetes and HCC in HBV carriers: at least in Far-East Asia, HBV-related HCC has an earlier onset than type 2 diabetes (55 years old vs 57 years old) and HCV-related HCC (65 years old).[42]

IMPACT ON THERAPEUTIC OUTCOMES

The impact of steatosis, IR, or clinical metabolic risk factors on the response to antiviral therapy in patients with HBV was insufficiently studied. Given the potency of current analogue therapies for HBV, this impact is probably marginal. Two studies[43,44] failed to show any deleterious impact of steatosis on the response to pegylated

interferon. Pivotal studies of pegylated interferon α-2a, entecavir, or tenofovir did not report reduced antiviral efficacy in patients with steatosis, overweight, or diabetes. A Chinese study claimed that the rates of HBV DNA clearance at 6, 12, and 24 months of treatment with entecavir were lower in patients with steatosis, high BMI, or high waist circumference.[45] In the absence of confirmation and of a strong pharmacologic rationale, the relevance of these findings is unclear. However, type 2 diabetes and obesity were significant negative predictors for the regression of cirrhosis in tenofovir-treated patients, despite similar HBV DNA control or HBsAg loss.[46]

SUMMARY

Despite some experimental data that show that the HB*x* protein can induce hepatic lipogenesis, clinical studies do not support the concept of viral-induced steatosis in CHB. Instead, HbsAg carriers diagnosed with steatosis usually have coexisting exposure to metabolic risk factors or alcohol consumption, as a result of the high prevalence of these conditions in the general population. Steatosis by itself is not associated with fibrosis or deleterious outcomes in CHB. In contrast, obesity, diabetes, and IR significantly increase the risk of advanced fibrosis, cirrhosis occurrence, and hepatocellular carcinoma in HBsAg carriers. These associations are often strongest for IR, suggesting that the latter is a major confounder for obesity and diabetes. In CHB, careful diagnosis of concurrent NAFLD and metabolic risk factors is therefore important for prognostic purposes. Future studies will need to address whether their correction benefits the long-term prognosis of HbsAg carriers.

DRUG-INDUCED LIVER DISEASE

Patients with NAFLD have numerous concurrent comorbidities (eg, diabetes, hypertension, dyslipidemia, hyperuricemia), which entail a large consumption of drugs, some of which have well-described liver toxicity. Overweight and obesity are the main risk factors of NAFLD, and it has been shown that obese individuals consume more drugs than those who are not obese.[47] The overall aging of the population and the relentless increase in the prevalence of obesity (and therefore NAFLD) are additional reasons why drug consumption in patients with NAFLD is increasing. Because of this high level of exposure of a sizable fraction of the general population, the question whether patients with NAFLD are at higher risk of drug-induced liver injury (DILI) than patients without NAFLD becomes highly relevant.

Over the past decade, it has become increasingly evident that an acute challenge in a steatotic liver results in extensive injury, more than results from the same challenge in a normal liver. In a landmark experiment, Diehl and colleagues[48] have shown that physiologic doses of lipopolysaccharide induce a higher increase in aminotransferase levels and higher levels of mortality in rodents with fatty liver than in those with normal liver. This vulnerability to necrosis was the result of the induction of proinflammatory cytokines and an increased susceptibility toward their actions but also a failure of compensatory regenerative mechanisms.[49] In a series of experiments, Feldstein and colleagues[50,51] have also shown that the steatotic liver is sensitized to apoptotic liver injury, a phenomenon that might be mediated by key regulators of lipid biosynthesis.[52] Others have shown that the steatotic liver is also more susceptible to bile acid–induced hepatotoxicity.[53] This higher susceptibility of the fatty liver to injury seems to occur irrespective of the experimental model of steatosis (genetic or dietary)[48,50,54] and irrespective of the noxious agent.[49,52,54] Preliminary human studies in patients with NAFLD have reported a dysfunctional replenishment of hepatic adenosine triphosphate stores after acute fructose challenge, which, arguably, could play a

role in propagating liver injury.[55] Several studies have documented enhanced hepatic expression and activity of cytochrome P450 subtype 2E1 in the liver of obese patients or patients with NAFLD[56–58] (reviewed in Ref.[59]), which, given the central role of this cytochrome in drug biotransformation, suggests a higher risk of drug-induced liver disease in patients with steatosis (reviewed in Ref.[60]).

However exciting these experimental data, available data in humans do not offer much credence to an overall increased risk of DILI in obese, diabetic, or NAFLD populations. Traditionally, DILI is classified into 2 broad categories: idiosyncratic DILI, the most common form of DILI (acute liver failure excepted) and intrinsic DILI.[61,62] Idiosyncratic DILI is unpredictable and does not depend directly on the dose (although some dose dependency exists); intrinsic DILI is dose dependent, therefore predictable.

Idiosyncratic DILI

There is little clinical evidence that NAFLD increases the risk of idiosyncratic DILI. The 2 prospective, population-based studies of the incidence of DILI[63,64] do not mention BMI, diabetes, any concurrent metabolic comorbidity, or NAFLD as a predisposing factor of DILI. Although in the French study, this particular association might not have been studied or available for study, in the Icelandic DILI population the prevalence of obesity and diabetes was low (10% and 5%, respectively).[64] Large prospective registries of reported DILI do not mention either increased odds of hepatotoxicity in people with NAFLD or in those sharing metabolic risk factors of NAFLD.[65] Tarantino and colleagues[66] suggested that patients with NAFLD are at higher risk of DILI than patients with HCV. However, their study is based on few events (6 cases of DILI among 74 patients with NAFLD and 1 case among 174 patients with HCV) and questionable methodology.

Although NAFLD or components of the metabolic syndrome do not seem to increase the incidence of idiosyncratic DILI, a distinct question is whether they are associated with a more severe course of DILI. There is some evidence suggesting that this might be the case for diabetes mellitus. In a large series prospectively collected by the US DILI Network, Chalasani and colleagues[67] reported that among the first 300 collected cases, the proportion of diabetes was higher in the severe DILI cases (37% vs 25% in mild to moderate DILI), whereas there were no differences in BMI. Diabetes increased the risk of severe DILI by an odds ratio of 2.7, independent of age, alcohol, and the pattern of liver injury (cytolytic or cholestatic). This observation could not be confirmed by a Scandinavian series of severe DILI, because data on metabolic risk factors were not reported.[68] El Serag and Everson[69] noted that veterans with type 2 diabetes had a 40% higher risk of developing acute liver failure, even after excluding patients with chronic liver disease, viral hepatitis, or congestive heart failure. It is not clear how much of this excess risk of acute liver failure could be related to DILI. However, this observation suggests that diabetes, either directly or not, might increase the risk of severe liver injury in cases of acute liver injury, possibly iatrogenic in nature. This hypothesis still awaits confirmation. The Acute Liver Failure Study group investigated a series of 308 consecutive cases of acute liver failure admitted in referral centers, but no information on metabolic risk factors versus clinical outcome is available.[70]

Intrinsic DILI

Acetaminophen

NAFLD might increase the risk of acetaminophen hepatotoxicity. Nguyen and colleagues[71] studied 42,781 admissions for acetaminophen overdose reported over 8 years in the Nationwide Inpatient Sample, covering 20% of US hospitals. Only 7% of patients developed acute liver injury. Patients diagnosed with NAFLD had 7.5 higher

odds of progressing to acute liver injury, independent of age, sex, or comorbidities.[71] This association was not driven by patients with cirrhosis. Although NAFLD increased the risk of acetaminophen-induced hepatitis, it did not increase the risk of severe liver failure (ie, acute liver injury with encephalopathy). However, the diagnosis of NAFLD was most certainly considerably underestimated in this series (as the 1.1% prevalence of HCV among patients with acetaminophen overdose vs only 0.1% prevalence of NAFLD suggests), because liver biopsy is usually not performed in patients with acetaminophen overdose. Nonetheless, this work based on hospitalization diagnosis codes is one of the few solid reports suggesting that NAFLD might increase the occurrence of acetaminophen-induced DILI. Experimental data have shown that obesity is associated with reduced glutathione levels,[72] a critical defense mechanism against hepatotoxic acetaminophen metabolites such as *N*-acetyl-p-benzoquinone imine (NAPQI). Also, CYP2E1 is responsible for NAPQI formation, and there are some data that hepatic CYP2E1 activity is enhanced in overweight individuals with NAFLD.[56,59] These experimental data might explain the clinical findings mentioned earlier.

Methotrexate

Methotrexate-induced hepatotoxicity consists of a chronic fibrogenic injury rather than an acute liver injury, typical of classic DILI. The potential for long-term liver fibrosis has been best described in patients receiving methotrexate for psoriasis, but in these patients the impact of NAFLD per se has not been studied. Because methotrexate can induce steatosis, the distinction between drug-induced and underlying, IR-induced steatosis is not a simple one. However, several studies have examined whether risk factors for NAFLD increase the risk of fibrosis in methotrexate-treated patients. Two studies clearly show that diabetes is associated with higher chances of developing liver fibrosis as well as advanced fibrosis.[73,74] One study[73] but not the other[74] found that overweight also contributes to fibrosis. Patients with both risk factors might develop advanced fibrosis at a higher rate and for a lower cumulative dose of methotrexate; fibrosis monitoring should be particularly stressed in these patients, preferably through noninvasive methods.[75,76]

Anesthetic drugs

It has been long recognized that halogenated anesthetic drugs such as halothane can induce acute hepatitis. The most severe form associates fever, rash, arthralgia, and eosinophilia and is believed to be caused by an immunoallergic mechanism, usually after repeated exposure. This particular form occurs frequently in obese individuals, although it is not well understood whether obesity specifically favors the hypersensitive reaction, because this has not been tested or reproduced in animal models.[77] In humans, the predominant mechanism of halothane biotransformation is oxidative, through cytochrome P4502E1. This situation results in a highly reactive intermediate metabolite, trifluoroacetyl chloride, which binds covalently to hepatocyte proteins and lipids and triggers an adaptive immune response. Obesity increases the activity of several P450 cytochromes, including CYP4502E1,[59] and this might account for a higher level of hepatotoxicity for halothane (and also for acetaminophen). Despite a lower rate of oxidative metabolism with isoflurane (0.2% compared with 30% with halothane), hepatotoxicity with isoflurane has still been described, including in an obese patient.[78] These events are too rare to infer any specific contribution of NAFLD, obesity, or diabetes in increasing the risk of isoflurane hepatitis. There is clearly cross-reactivity between halothane, isoflurane, and enflurane, to the extent that previous exposure to one of these drugs may increase the risk of hepatotoxicity for another compound.[79–82]

SUMMARY

Unsurprisingly for a disease process that is unpredictable, rare, and not dose related, intrinsic DILI does not seem to increase with NAFLD or associated metabolic risk factors. There is some clinical evidence that obese or diabetic patients with NAFLD might be at higher risk of hepatotoxicity from acetaminophen and at higher fibrotic risk from methotrexate. Because most registries of DILI did not specifically consider NAFLD or the metabolic syndrome as risk factors for hepatotoxicity, future studies will be critical for testing this association. Nonetheless, the contrast between the abundance of experimental data in favor of a heightened risk of hepatotoxicity in the fatty liver and the scarcity of human observations of DILI in patients with NAFLD point to a complex interplay between deleterious and protective mechanisms of cytotoxicity in human hepatic steatosis.

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